REMARKS

Claims 1-18, 20-25, 27 and 29-42 are currently pending, claims 1, 21 and 23 having been amended. New claim 43 has been added.

Claim Amendments

Claims 1, 21 and 23 have been amended to recite that the lipid phase is non-polar. Support for this amendment may be found throughout the specification and at least at page 9, line 8 and page 12, line 12 to page 13, line 11. Entry and consideration of the amendment are respectfully requested.

§ 103(a) Rejection - Gordon in view of Farmer

Claims 1-18, 20-25, 27 and 29-42 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Gordon (USPN 5,763,332) in view of Farmer (WO 01/13956). Applicants respectfully traverse this rejection.

The Examiner has asserted a rejection based on Gordon in view of Farmer. Gordon allegedly teaches a cleansing wipe with a high internal phase inverse emulsion. Farmer allegedly teaches a towelette with Emu Oil and lactic-acid producing bacteria.

However, the Examiner has failed to allege a combination that will result in the presently claimed invention. For at least this reason the rejection is improper.

--1--

Claims 1, 21 and 23 (and new claim 43) recite, *inter alia*, that the lipid phase is non-polar.

Gordon relates to a high internal phase inverse emulsion. The high internal phase inverse emulsion of Gordon has three components (1) a continuous solidified external lipid; (2) an emulsifier that forms the emulsion when the external lipid phase is fluid; and (3) an ***internal polar phase*** dispersed in the external lipid phase. See column 11, lines 62-65.

Accordingly, the proposed combination relies upon an emulsion with an internal polar phase. However, the presently claimed invention is directed to a non-polar lipid phase. Accordingly, the combination of Gordon and Farmer does not teach or suggest the presently claimed invention.

--2--

As noted above, Gordon relates to a high internal phase inverse emulsion. Farmer relates to a towelette with Emu Oil and lactic-acid producing bacteria. One skilled in the art would not have been motivated to incorporate the Emu Oil/bacteria preparation as part of the high internal phase inverse emulsion of Gordon. The Examiner has noted that the high internal phase inverse emulsion of Gordon comprises waxy lipids and such. The Examiner appears to rely on this disclosure to assert one would be motivated to incorporate the Emu Oil/bacteria preparation of Farmer into the high internal phase inverse emulsion of Gordon. However, in making this modification, the Examiner has made an oversight. One skilled in the art would not be motivated to incorporate an Emu Oil with lactic-acid producing bacteria in the high internal phase inverse emulsion of Gordon.

The high internal phase inverse emulsion of Gordon has three components (1) a continuous solidified external lipid; (2) an emulsifier that forms the emulsion when the external lipid phase is fluid; and (3) an internal polar phase dispersed in the external lipid phase. See column 11, lines 62-65. The internal polar phase is disclosed to be preferably water or, alternatively, an alcohol or glycol. See column 13, line 52 to column 14, line 59. For the asserted combination to have the claim water activity (see discussion below), the internal polar phase cannot be water. One skilled in the art would understand that (non-water) embodiments disclosed in Gordon for the internal polar phase would be harmful to the lactic acid producing bacteria.

The Examiner has asserted that this argument is not substantiated by facts. Accordingly, applicants include, as attachments, references that support the statement that the internal polar phase would be harmful to the lactic acid producing bacteria.

Attachment 1: *McDonnell G, Russell AD (1999),* "Antiseptics and disinfectants: activity, action, and resistance". Clin. Microbiol. Rev. 12 (1): 147–79. PMID 9880479.

Attachment 2: J Chirife et al., Antimicrob Agents Chemother. 1983 September; 24(3): 409–412, In vitro antibacterial activity of concentrated polyethylene glycol 400 solutions.

Attachment 3: Kinnunen T, Koskela M, Acta Derm Venereol, 1991;71(2):148-50, Antibacterial and antifungal properties of propylene glycol, hexylene glycol, and 1,3-butylene glycol in vitro.

Even a quick review of these references should establish for the Examiner's benefit that the arguments are substantiated by facts. Accordingly, the Examiner must give weight to the statement that one skilled in the art would understand that the (non-water) embodiments disclosed in Gordon for the internal polar phase would be harmful to the lactic acid producing bacteria

The internal polar phase (of the high internal phase inverse emulsion) of Gordon would be harmful to the lactic acid producing bacteria. Thus, one skilled in the art reading Gordon would not be motivated to incorporate a lactic acid producing bacteria in the high internal phase inverse emulsion of Gordon. Accordingly, one skilled in the art would not combine Gordon and Farmer in the manner asserted by the Examiner.

--3--

Even if one skilled in the art were to combine modify Gordon in order to incorporate the Emu Oil with lactic-acid producing bacteria of Farmer, there is still no teaching or suggestion found in either Gordon or Farmer that relates to the claimed tissue with separate parts, one of which has a cleaning liquid and another with a preparation of lactic acid producing bacteria and a lipid phase, the preparation having a water activity of 0.30 or less.

The Examiner asserts "that said activity would have been inherent to the lactobacillus plantarum of WO '956 because said lactobacillus would comprise this characteristic." However, the water activity is a measurement of water content of a mixture/composition/preparation - not simply a bacterium. The feature at issue is the claim recitation directed to the ***preparation*** of one or more lactic acid producing bacterial strains having a water activity of 0.30 or less. Accordingly, the Examiner has not made a proper allegation regarding the ***preparation***. A proper *prima facie* case of obviousness has not been presented.

--4--

Claim 1 recites, *inter alia*, a hygiene tissue that has two parts. A first part has a cleaning liquid. A second part has a preparation of a lactic acid producing bacterial strain and a lipid phase, the preparation having a water activity of 0.30 or less. The first and second parts are separate in different parts of the tissue. The first and second parts are separated by a barrier

The Examiner appears to rely on a disclosure in Gordon of "Instead, these HLB detergent surfactants can be applied or included in the article separately from the emulsion." (The Examiner cites to column 17, lines 45-65 of Gordon). However, the Examiner has still failed to allege or assert a teaching of a ***barrier***. A review of Gordon does not reveal a teaching of a combination that includes a barrier. Instead, the "separate" parts in Gordon are disclosed to be simply one part "applied to the carrier either before or after application of the emulsion to the carrier." Column 17, line, 67 to column 18, line 1. Accordingly, a combination that includes a barrier has not been disclosed or suggested.

Further, claim 21 recites, *inter alia*, that the first part and the second part are separate sheets joined together along two opposite side edges. And, claim 23 recites, *inter alia*, that the second part comprises a first sheet and a second sheet joined together along two opposite side edges and the first part comprises a sheet joined to one of said opposite side edges of the second part and extending along said side edge with one of a side edge of the sheet of the first part

A combination that includes each feature of claim 21 or claim 23 has not been disclosed or suggested. Further, the Examiner has failed to allege these combinations. The Examiner is respectfully requested to specifically identify where in Gordon (or other cited art) disclosure of the above-identified claim recitations may be found. Applicants respectfully assert that a proper *prima facie* case of obvious has not been presented.

--Conclusion--

The Examiner has failed to allege a combination that will result in the presently claimed invention.

The proposed combination relies upon an emulsion with an internal polar phase. However, the presently claimed invention is directed to a non-polar lipid phase.

The internal polar phase (of the high internal phase inverse emulsion) of Gordon would be harmful to the lactic acid producing bacteria. Accordingly, one skilled in the art would not combine Gordon and Farmer in the manner asserted by the Examiner.

The asserted art does not teach or suggest a preparation of lactic acid producing bacteria having a water activity of 0.30 or less.

A combination that includes a barrier, or other claimed structural features, has not been disclosed or suggested.

Accordingly, the rejection of claims 1-18, 20-25, 27 and 29-42 as being unpatentable over Gordon (USPN 5,763,332) in view of Farmer (WO 01/13956) is respectfully requested to be withdrawn.

New Claim 43

New claim 43 is support by the original claims and throughout the specification. New claim 43 is nearly identical to claim 1, claim 43 not including the recitation that the first and second parts are separated by a barrier. Claim 43 is at least patentable over the cited art for reasons similar to the reasons claim 1 is patentable.

Conclusion

For the reasons stated above, it is requested that all the rejections be withdrawn and that this application be allowed in a timely manner.

Should any questions arise in connection with this application or should the Examiner feel that a teleconference with the undersigned would be helpful in resolving any issues pertaining to this application, it is requested that the undersigned be contacted at the number indicated below.

Respectfully submitted,

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Date: October 24, 2007

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1: Acta Derm Venereol. 1991;71(2):148-50.

Links

Antibacterial and antifungal properties of propylene glycol, hexylene glycol, and 1,3-butylene glycol in vitro.

Kinnunen T, Koskela M.

Department of Dermatology, University of Oulu, Finland.

The antimicrobial properties of three glycols, - propylene glycol, hexylene glycol, and 1,3-butylene glycol - against Candida albicans, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes A, Streptococcus mitis, and E. coli were studied in vitro. Within 20 h, 10% and 30% hexylene glycol in fresh tryptic soy broth were able to kill all the micro-organisms listed above. Five percent hexylene glycol showed some antimicrobial properties but the 1% agent had no effect. Thirty percent 1,3-butylene glycol and 30% propylene glycol were approximately as effective as 10% HG. The results speak in favour of using hexylene glycol in cosmetic and dermatological vehicles instead of propylene glycol and 1,3-butylene glycol.

PMID: 1675525 [PubMed - indexed for MEDLINE]

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Vol. 24, No. 3

In Vitro Antibacterial Activity of Concentrated Polyethylene Glycol 400 Solutions

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It was found that concentrated polyethylene glycol 400 (PEG 400) solutions have significant antibacterial activity against various pathogenic bacteria, including *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus*. This effect might be attributed to two effects: lowering of water activity and, superimposed on this, the specific action of PEG-400 molecules on bacterial cells. Phase-contrast microscopic observations of cells placed in contact with PEG 400 revealed clumping and morphological changes of bacterial cells. The larger changes in appearance were evidenced by the species which were more rapidly killed by PEG 400. The results obtained suggested that concentrated PEG 400 solutions may have a potential value in medicine as a topical antibacterial agent. The feasibility of this application is the subject of present investigation.

In the past years, various studies have been made on the relation of microorganisms to high solute concentrations. These have included studies of growth inhibition mechanisms, the solute concentration needed to inhibit growth, and the physiological basis of microorganisms that can withstand such high solute concentrations (1, 3, 9, 10). It has been observed that the water requirements of bacteria as well of other microorganisms are best expressed in terms of water activity (a_w) rather than water concentration. Water activity is given as $a_w = p/p_0$ where p is the vapor pressure of water in solution and p_0 is the vapor pressure of pure water at the same temperature. At present, numerous data are available on the relation between a_w and the ability of microorganisms to grow (9). However, it has also established that the a_w of the medium is not the only determining factor regulating the biological response of bacteria; the nature of the solute used to reduce a_w is also important (5).

The bactericidal activity of a variety of glycols has been studied by Robertson et al. (14). They investigated the bactericidal action in vitro of a number of glycols for pneumococci, hemolytic streptococci, and staphylococci. Three glycols were studied more extensively than the others, namely, propylene, dipropylene, and triethylene. Olitzky (12) also reported on the germicidal efficiency of concentrated propylene glycol solutions. Plitman et al. (13) investigated the bacteriostatic and bactericidal activity of several diols, employing *Staphylococcus aureus* as test organism.

Recently, Vaamonde et al. (15) showed that polyethylene glycol 400 (PEG 400) appeared to have a significant inhibitory effect on one strain of *S. aureus*, independent of a_w lowering. The present study describes investigations that were carried out to explore the bactericidal effect of concentrated PEG 400 against various pathogenic bacteria relevant to infected wounds and other superficial lesions. The bacterial species studied included *S. aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Escherichia coli*. To our knowledge, no detailed study of the bactericidal activity of concentrated PEG 400 solutions on these bacteria has been reported.

MATERIALS AND METHODS

Microorganisms. The sources of the strains used in this study and their strain numbers are as follows: *S. aureus* ATCC 6538P; *P. aeruginosa* ATCC 27853; *K. pneumoniae* ATCC 10031; *E. coli* ATCC 25922; *S. aureus* 41/82, a wild-type strain obtained from Hospital Muñiz, Buenos Aires, Argentina; *P. aeruginosa* 15/4, *K. pneumoniae* 6440, and *E. coli* 11197, strains obtained from the culture collection of Instituto Nacional de Microbiología "C. G. Malbrán".

Media. Brain heart infusion (BHI) broth (Oxoid Ltd., London, England) was used for all tests. PEG 400 (Mallinckrodt Chemical Works, St. Louis, Mo.) was added to BHI before sterilization. After dissolution of PEG 400, the pH was adjusted to 7.0 by the addition of 8 N HCl. The media was autoclaved with precautions to avoid loss of water by vaporization which could change the concentration of PEG 400. In most experiments the concentration of PEG 400 in the media was adjusted to 1.6 g of PEG 400 per g of water;

in some experiments a concentration of 4.8 g of PEG 400 per g of water was utilized.

Technique of tests. Growth inhibition studies were made in 250-ml screw-top glass bottles containing about 18 g of inoculated medium containing PEG 400 which were incubated at 35°C in a constant-temperature cabinet. The bacteria used for the inoculum were derived from a culture in the log phase.

Enumeration procedure. Counts were determined by the use of plate count agar (Difco Laboratories, Detroit, Mich.) for *S. aureus*, *K. pneumoniae*, and *E. coli*; CLED medium with Andrade indicator (Oxoid Ltd.) was used for *P. aeruginosa*. The samples were serially diluted (1:10) with 0.1% peptone (Oxoid Ltd.) before plating; the volume plated was 0.1 ml. The plates were incubated at 35°C for 24 to 48 h, and the colonies were counted.

Phase-contrast microscopy. Observations were carried out at room temperature with a dark contrast-phase Nikon Labophot microscope. A CF acromat 100/1.25 DLL objective and a CF Photo ocular 5× projective (field number 20) were used as an optical recording combination. Photomicrographs (at magnifications of 500 on the negative) were taken with a Nikkon FX Microflex 35-mm manual camera on Ilford PAN F film exposed as 18 DIN (50 ASA).

Cells to be observed and photographed were obtained from BHI broth cultures in logarithmic growth phase which were centrifuged at 20 × *g*. The pellets were resuspended in fresh culture medium with PEG 400 by using a vortex mixer. The amount of former medium (about 0.2 ml) was taken into account to obtain the final PEG 400 concentrations as well as for the determination of the number of cells per milliliter useful for microscope sampling observations (about 10^6 to 10^8 cells per ml). Samples were removed from media and placed on microscope slides with a calibrated loop (10^{-3} ml) at various times after the cells were subjected to PEG 400 solutions (1.6 g of PEG 400 per g of water in broth).

Water activity determination. The water activity of media containing PEG 400 was determined by using a fiber-dimensional hygrometer, a_w-Wert Messer, manufactured by Firma Lufft, Stutgart, West Germany (2).

RESULTS

Survival of colony-forming ability. Figure 1 shows the behavior of *S. aureus* ATCC 6538 P, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 10031 in BHI media with PEG 400 (1.6 g of PEG 400 per g of water in medium). Initial inoculum levels of all microorganisms were fixed at 10^7 to 10^8 CFU/ml. It can be seen that viable cells of *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 10031 declined rapidly, leading to almost total loss of viable population in 10 to 14 h of incubation. *S. aureus* ATCC 6538 P was far more resistant than the other bacteria studied, although the viable population also declined continuously; almost total destruction of cells was achieved after about 120 h of incubation. Results similar to those in Fig. 1 were obtained with *E. coli* 11179, *K. pneumoniae* 6440, and *S. aureus* 41/82. It is noteworthy that control experiments

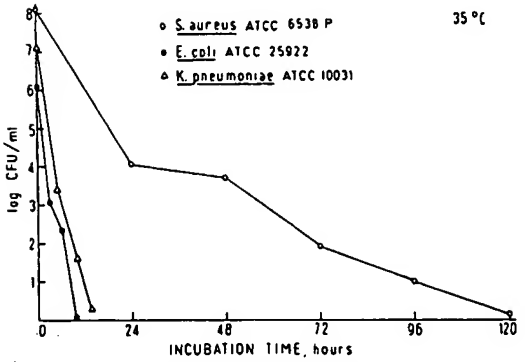

FIG. 1. Survival of colony-forming ability of *S. aureus* ATCC 6538 P, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 10031 in PEG 400 added to BHI medium (1.6 g of PEG 400 per g of water in broth) incubated at 35°C.

(BHI broth without PEG 400) were also performed (not shown) and showed that all species remained at 10^8 to 10^9 CFU/ml during the period of incubation.

Figure 2 shows the behavior of *P. aeruginosa* ATCC 27853 and 15/4 in BHI broth with PEG 400 added to the same level of concentration as before. It can be seen that PEG 400 kills the cells rapidly as compared to other species studied; almost complete inactivation of cells occurred at 2 to 4 h of incubation.

Additional survival experiments were performed by using a more concentrated solution of PEG 400; in this case a value of 4.8 g of PEG 400 per g of water in medium was adopted. It was found that complete destruction of cells of *P. aeruginosa* ATCC 27853 (initial inoculum, 3.8 × 10^8 CFU/ml), *P. aeruginosa* 15-4 (initial inoculum, 6.8 × 10^8 CFU/ml), and *E. coli* 11197 (initial inoculum, 3.7 × 10^8 CFU/ml) occurred in

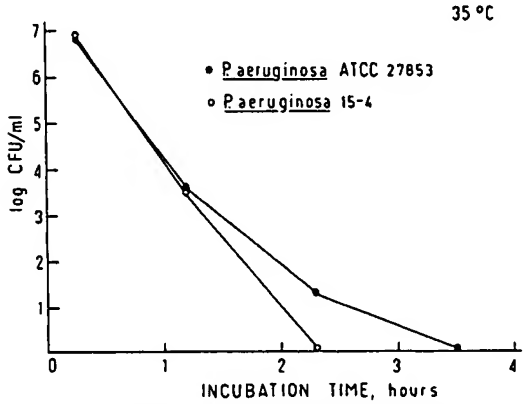

FIG. 2. Survival of colony-forming ability of *P. aeruginosa* ATCC 27853 and 15/4 in PEG 400 added to BHI medium (1.6 g of PEG 400 per g of water in broth) incubated at 35°C.

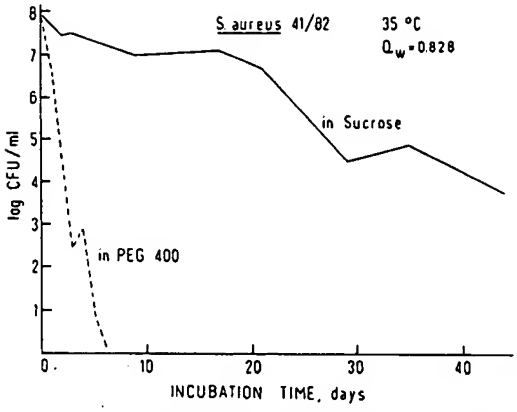

FIG. 3. Comparison of survival curves of *S. aureus* 41/82 in BHI broth of a_w lowered by the addition of PEG 400 or sucrose and incubated at 35°C.

only 20 to 25 min of incubation at 35°C.

An attempt was made to distinguish between the effects of lowered a_w and specific solute effects on bacteria, as follows. Figure 3 compares the survival curves of *S. aureus* 41/82 in BHI broth with the a_w lowered to the same value (around 0.828) by the addition of either PEG 400 or sucrose (220 g of sucrose per 100 g of water); data for the sucrose solution were taken from Chirife et al. (4). Sucrose was used for comparison since it is also a nonionic solute of similar molecular weight. It can be seen that the bactericidal action of PEG 400 is dramatically different (faster) than that of sucrose. Similar results (not shown) were obtained when comparing the antibacterial activities of PEG 400 and sucrose against *E. coli*, *P. aeruginosa*, or *K. pneumoniae* in solutions of equal a_w.

Microscopic observations. Cells of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* were observed under a phase-contrast microscope 1 to 2 min after being placed in contact with PEG 400 (1.6 g of PEG 400 per g of water in medium); in some cases the observations were made after 15 min. The phase-contrast microscopic observations indicated that in all cases PEG 400 caused immediate clumping of the cells. This is illustrated in Fig. 4, which shows normal log-phase cells of *K. pneumoniae* 6440 (Fig. 4A) and the same cells shortly after being placed in broth with PEG 400 (Fig. 4B). Similar morphological changes were observed for the other bacteria studied. It is noteworthy, however, that in the case of *S. aureus* the morphological modifications (clump formation) were less evident than for the other cells.

DISCUSSION

It is generally accepted that when a solute such as sucrose is used to reduce the a_w, the a_w itself is the main determining factor regulating the biological response of bacteria. Bacterial cells are largely impermeable to sucrose (8), and lowered a_w withdraws water from the cell and may cause plasmolysis. However, it is apparent from the results in Fig. 3 that PEG 400 produces two effects: lowering of a_w and, superimposed

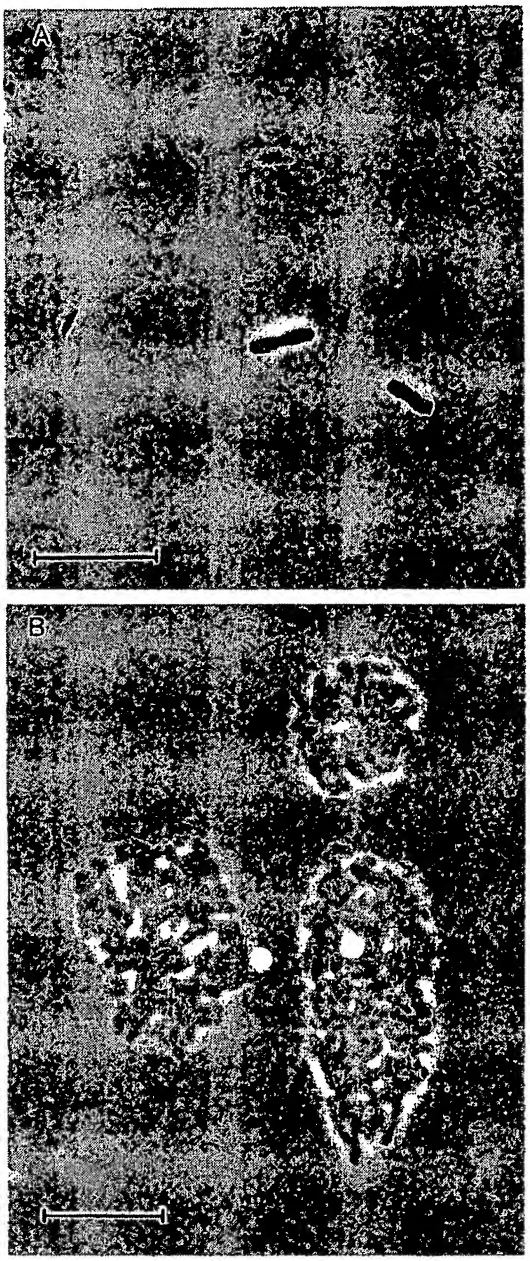

FIG. 4. Phase-contrast micrographs of cells of *K. pneumoniae* ATCC 10031. (A) Normal log-phase cells; (B) log-phase cells shortly after being placed in broth with PEG 400. Bar, 10 μm.

on this, the specific action of its molecules on bacterial cells.

PEG 400 appears to cause drastic changes in cell morphology within minutes after addition to the cells. However, we cannot say that these effects (clumping) are directly related to the decrease in bacterial count in the PEG 400-containing cultures due to the relatively slow effect observed on bacterial survival (Fig. 1). We observed, however, that the larger changes in morphology were evidenced by the species which were more rapidly killed by PEG 400, i.e., *K. pneumoniae* and *P. aeruginosa*. As previously mentioned, clumping of the cells was less conspicuous in *S. aureus* (Fig. 1), which was far more resistant than the other bacteria.

The results obtained here suggest that concentrated PEG 400 solutions may have a potential value in medicine as a topical antibacterial agent. Further studies are under way on the rate at which killing of bacteria could be produced by more concentrated solutions, i.e., above 4.8 g of PEG 400 per g of water, as well as on the activity of PEG 400 in the presence of serum or albumin. Information on these latter points is of particular importance in relation to the effectiveness of PEG 400 as a disinfecting agent, since we are interested in a compound which is rapidly bactericidal. Of course, the killing effect of PEG 400 is much less than that of traditional disinfecting agents. However, its merits should be judged, considering that PEG 400 meets many of the physicochemical requirements for an ideal disinfectant for topical use. PEG 400 is neutral, odorless, highly soluble in water (soluble in all proportions), and nonirritating to the skin, does not decompose, has a low vapor pressure, and its toxicity from acute oral administration or topical application is low (6, 11). At present PEG 400 (as well as other polyethylene glycols) has several pharmaceutical applications not due to its antibacterial properties. Polyethylene glycols find their chief application in the preparation of hydrophylic ointment bases incorporating a wide variety of therapeutic materials, including antibacterials, antibiotics, and steroids. PEG 400 may be also safely used in foods as a coating, binder, or plasticizing or bodying agent (7).

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Brown, A. D.** 1976. Microbial water stress. Bacteriol. Rev. 40:803–846.
2. **Chirife, J., and C. Ferro Fontán.** 1980. The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. V. Experimental investigation of the a_w lowering behavior of sodium lactate and some related compounds. J. Food Sci. 45:802–804.
3. **Chirife, J., C. Ferro Fontán, and O. C. Scorza.** 1981. The intracellular water activity of bacteria in relation to the water activity of the growth medium. J. Appl. Bacteriol. 50:475–479.
4. **Chirife, J., L. Herszage, A. Joseph, and E. S. Kohn.** 1983. In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in the treatment of infected wounds. Antimicrob. Agents Chemother. 23:766–773.
5. **Christian, J. H. B.** 1981. Specific solute effects on microbial water relations, p. 825–854. *In* L. B. Rockland and G. F. Stewart (ed.), Water activity: influences on food quality. Academic Press, Inc., New York.
6. **Dow Chemical U.S.A.** 1974. Polyethylene glycols handbook. Dow Chemical Co., Midland, Mich.
7. **Furia, T. E.** 1975. Handbook of food additives, 2nd ed. CRC Press, Cleveland, Ohio.
8. **Gould, G. W., and J. C. Measures.** 1977. Water relations in single cells. Philos. Trans. R. Soc. London Ser. B. 278:151–166.
9. **Kushner, D. J.** 1971. Influence of solutes and ions on microorganisms, p. 259–283. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Inc., London.
10. **Kushner, D. J.** 1978. Life in high salt and solute concentrations: halophilic bacteria, p. 317–368. *In* D. J. Kushner (ed.), Microbial life in extreme environments. Academic Press, Inc., New York.
11. **Lockard, J. S., R. H. Levy, W. C. Congdom, and L. L. DuCharme.** 1979. Efficacy and toxicity of the solvent polyethylene glycol 400 in monkey model. Epilepsia 20:77–84.
12. **Olitzky, I.** 1965. Antimicrobial properties of a propylene glycol based topical therapeutic agent. J. Pharm. Sci. 54:787–788.
13. **Plitman, M., Y. Park, R. Gómez, and A. J. Sinskey.** 1973. Viability of *Staphylococcus aureus* in intermediate moisture meats. J. Food Sci. 38:1004–1008.
14. **Robertson, O. H., E. M. Appel, T. T. Puck, H. M. Lemon, and M. H. Ritter.** 1948. A study of the bactericidal activity in vitro of certain glycols and closely related compounds. J. Infect. Dis. 83:124–137.
15. **Vaamonde, G., J. Chirife, and O. C. Scorza.** 1982. An examination of the minimal water activity for *Staphylococcus aureus* ATCC 6538 P growth in laboratory media adjusted with less conventional solutes. J. Food Sci. 47:1259–1262.

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0893-8512/99/$04.00+0

Antiseptics and Disinfectants: Activity, Action, and Resistance

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INTRODUCTION

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections (277, 454). Mounting concerns over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased use of antiseptics and disinfectants by the general public. A wide variety of active chemical agents (or "biocides") are found in these products, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation (39). Despite this, less is known about the mode of action of these active agents than about antibiotics. In general, biocides have a broader spectrum of activity than antibiotics, and, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets. The widespread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance, in particular cross-resistance to antibiotics. This review considers what is known about the mode of action of, and mechanisms of microbial resistance to, antiseptics and disinfectants and attempts, wherever possible, to relate current knowledge to the clinical environment.

A summary of the various types of biocides used in antiseptics and disinfectants, their chemical structures, and their clinical uses is shown in Table 1. It is important to note that many of these biocides may be used singly or in combination in a variety of products which vary considerably in activity against microorganisms. Antimicrobial activity can be influenced by many factors such as formulation effects, presence of an organic load, synergy, temperature, dilution, and test method. These issues are beyond the scope of this review and are discussed elsewhere (123, 425, 444, 446, 451).

DEFINITIONS

"Biocide" is a general term describing a chemical agent, usually broad spectrum, that inactivates microorganisms. Because biocides range in antimicrobial activity, other terms may be more specific, including "-static," referring to agents which inhibit growth (e.g., bacteriostatic, fungistatic, and sporistatic) and "-cidal," referring to agents which kill the target organism (e.g., sporicidal, virucidal, and bactericidal). For the purpose of this review, antibiotics are defined as naturally occurring or synthetic organic substances which inhibit or destroy selective bacteria or other microorganisms, generally at low concentrations; antiseptics are biocides or products that destroy or inhibit the growth of microorganisms in or on living tissue (e.g. health care personnel handwashes and surgical scrubs); and disinfectants are similar but generally are products or biocides that are used on inanimate objects or surfaces. Disinfectants can be sporostatic but are not necessarily sporicidal.

Sterilization refers to a physical or chemical process that completely destroys or removes all microbial life, including spores. Preservation is the prevention of multiplication of microorganisms in formulated products, including pharmaceuticals and foods. A number of biocides are also used for cleaning purposes; cleaning in these cases refers to the physical removal of foreign material from a surface (40).

MECHANISMS OF ACTION

Introduction

Considerable progress has been made in understanding the mechanisms of the antibacterial action of antiseptics and disinfectants (215, 428, 437). By contrast, studies on their modes of action against fungi (426, 436), viruses (298, 307), and protozoa (163) have been rather sparse. Furthermore, little is known about the means whereby these agents inactivate prions (503).

Whatever the type of microbial cell (or entity), it is probable that there is a common sequence of events. This can be envisaged as interaction of the antiseptic or disinfectant with the cell surface followed by penetration into the cell and action at the target site(s). The nature and composition of the surface vary from one cell type (or entity) to another but can also alter as a result of changes in the environment (57, 59). Interaction at the cell surface can produce a significant effect on viability (e.g. with glutaraldehyde) (374, 421), but most antimicrobial agents appear to be active intracellularly (428, 451). The outermost layers of microbial cells can thus have a significant effect on their susceptibility (or insusceptibility) to antiseptics and disinfectants; it is disappointing how little is known about the passage of these antimicrobial agents into different types of microorganisms. Potentiation of activity of most biocides may be achieved by the use of various additives, as shown in later parts of this review.

In this section, the mechanisms of antimicrobial action of a range of chemical agents that are used as antiseptics or disinfectants or both are discussed. Different types of microorganisms are considered, and similarities or differences in the nature of the effect are emphasized. The mechanisms of action are summarized in Table 2.

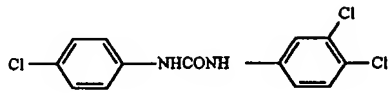
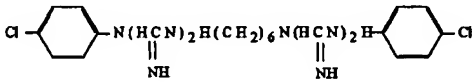
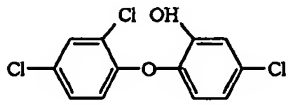
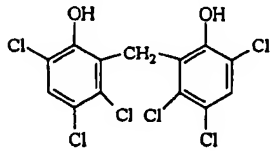
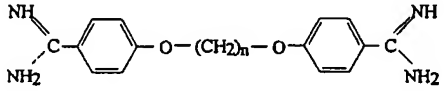
General Methodology

A battery of techniques are available for studying the mechanisms of action of antiseptics and disinfectants on microorganisms, especially bacteria (448). These include examination of uptake (215, 428, 459), lysis and leakage of intracellular constituents (122), perturbation of cell homeostasis (266, 445), effects on model membranes (170), inhibition of enzymes, electron transport, and oxidative phosphorylation (162, 272), interaction with macromolecules (448, 523), effects on macromolecular biosynthetic processes (133), and microscopic examination of biocide-exposed cells (35). Additional and useful information can be obtained by calculating concentration exponents (*n* values [219, 489]) and relating these to membrane activity (219). Many of these procedures are valuable for detecting and evaluating antiseptics or disinfectants used in combination (146, 147, 202, 210).

Similar techniques have been used to study the activity of antiseptics and disinfectants against fungi, in particular yeasts. Additionally, studies on cell wall porosity (117–119) may provide useful information about intracellular entry of disinfectants and antiseptics (204–208).

Mechanisms of antiprotozoal action have not been widely investigated. One reason for this is the difficulty in culturing some protozoa (e.g., *Cryptosporidium*) under laboratory conditions. However, the different life stages (trophozoites and cysts) do provide a fascinating example of the problem

TABLE 1. Chemical structures and uses of biocides in antiseptics and disinfectants

Alcohols	Ethanol	$CH_3—CHOH$	Antisepsis
	Isopropanol	$CH_3—CHOH—CH_3$	Disinfection
			Preservation
Aldehydes	Glutaraldehyde	$OH—CCH_2CH_2CH_2C—HO$	Disinfection
	Formaldehyde	$H—C—HO$	Sterilization
			Preservation
Anilides	General structure	$C_6H_5.NH.COR$	Antisepsis
	Triclocarban		
Biguanides	Chlorhexidine		Antisepsis
			Antiplaque agents
	Alexidine, polymeric biguanides		Preservation
			Disinfection
Bisphenols	Triclosan		Antisepsis
	Hexachlorophene		Antiplaque agents
			Deodorants
			Preservation
Diamidines	Propamidine		Antisepsis
			Preservation
	Dibromopropamidine		

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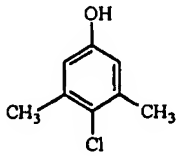
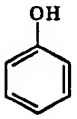
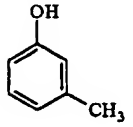
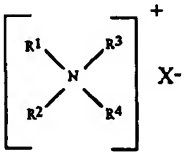
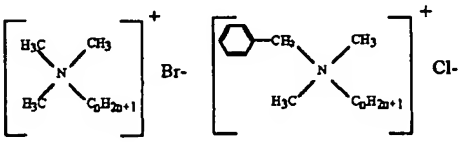
of how changes in cytology and physiology can modify responses to antiseptics and disinfectants. Khunkitti et al. (251–255) have explored this aspect by using indices of viability, leakage, uptake, and electron microscopy as experimental tools.

Some of these procedures can also be modified for studying effects on viruses and phages (e.g., uptake to whole cells and viral or phage components, effects on nucleic acids and proteins, and electron microscopy) (401). Viral targets are

TABLE 1—*Continued*

Halogen-releasing agents	Chlorine compounds	◇OCl-, HOCl, Cl_2	Disinfection Antisepsis
	Iodine compounds	◇I_2	Cleaning
Halophenols	Chloroxylenol (PCMX)		Antisepsis Preservation
Heavy metal derivatives	Silver compounds	Ag	Preservation Antisepsis
	Mercury compounds	Hg	Disinfection
Peroxygens	Hydrogen peroxide	H_2O_2	Disinfection
	Ozone	O_3	Sterilization
	Peracetic acid	$CH_3.COOOH$	
Phenols and cresols	Phenol		Disinfection Preservation
	Cresol		
Quaternary ammonium compounds	General structure		Disinfection Antisepsis Preservation
	Cetrimide, benzalkonium chloride		Cleaning

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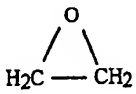
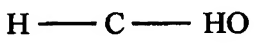
predominantly the viral envelope (if present), derived from the host cell cytoplasmic or nuclear membrane; the capsid, which is responsible for the shape of virus particles and for the protection of viral nucleic acid; and the viral genome. Release of an intact viral nucleic acid into the environment following capsid destruction is of potential concern since some nucleic acids are infective when liberated from the capsid (317), an aspect that must be considered in viral disinfection. Important considerations in viral inactivation are dealt with by Klein and Deforest (259) and Prince et al.

TABLE 1—*Continued*

Vapor phase	Ethylene oxide		Sterilization Disinfection
	Formaldehyde		
	Hydrogen peroxide	H_2O_2	

(384), while an earlier paper by Grossgebauer is highly recommended (189).

Alcohols

Although several alcohols have been shown to be effective antimicrobials, ethyl alcohol (ethanol, alcohol), isopropyl alcohol (isopropanol, propan-2-ol) and *n*-propanol (in particular in Europe) are the most widely used (337). Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal. They are, however, known to inhibit sporulation and spore germination (545), but this effect is reversible (513). Because of the lack of sporicidal activity, alcohols are not recommended for sterilization but are widely used for both hard-surface disinfection and skin antisepsis. Lower concentrations may also be used as preservatives and to potentiate the activity of other biocides. Many alcohol products include low levels of other biocides (in particular chlorhexidine), which remain on the skin following evaporation of the alcohol, or excipients (including emollients), which decrease the evaporation time of the alcohol and can significantly increase product efficacy (68). In general, isopropyl alcohol is considered slightly more efficacious against bacteria (95) and ethyl alcohol is more potent against viruses (259); however, this is dependent on the concentrations of both the active agent and the test microorganism. For example, isopropyl alcohol has greater lipophilic properties than ethyl alcohol and is less active against hydrophilic viruses (e.g., poliovirus) (259). Generally, the antimicrobial activity of alcohols is significantly lower at concentrations below 50% and is optimal in the 60 to 90% range.

Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water, it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis (278, 337). This is supported by specific reports of denaturation of *Escherichia coli* dehydrogenases (499) and an increased lag phase in *Enterobacter aerogenes*, speculated to be due to inhibition of metabolism required for rapid cell division (101).

Aldehydes

Glutaraldehyde. Glutaraldehyde is an important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment and as a fixative in electron

TABLE 2. Summary of mechanisms of antibacterial action of antiseptics and disinfectants

Target	Antiseptic or disinfectant	Mechanism of action
Cell envelope (cell wall, outer membrane)	Glutaraldehyde	Cross-linking of proteins
	EDTA, other permeabilizers	Gram-negative bacteria: removal of Mg^{2+}, release of some LPS
Cytoplasmic (inner) membrane	QACs	Generalized membrane damage involving phospholipid bilayers
	Chlorhexidine	Low concentrations affect membrane integrity, high concentrations cause congealing of cytoplasm
	Diamines	Induction of leakage of amino acids
	PHMB, alexidine	Phase separation and domain formation of membrane lipids
	Phenols	Leakage; some cause uncoupling
Cross-linking of macromolecules	Formaldehyde	Cross-linking of proteins, RNA, and DNA
	Glutaraldehyde	Cross-linking of proteins in cell envelope and elsewhere in the cell
DNA intercalation	Acridines	Intercalation of an acridine molecule between two layers of base pairs in DNA
Interaction with thiol groups	Silver compounds	Membrane-bound enzymes (interaction with thiol groups)
Effects on DNA	Halogens	Inhibition of DNA synthesis
	Hydrogen peroxide, silver ions	DNA strand breakage
Oxidizing agents	Halogens	Oxidation of thiol groups to disulfides, sulfoxides, or disulfoxides
	Peroxygens	Hydrogen peroxide: activity due to from formation of free hydroxy radicals (·OH), which oxidize thiol groups in enzymes and proteins; PAA: disruption of thiol groups in proteins and enzymes

TABLE 3. Mechanism of antimicrobial action of glutaraldehyde

Target microorganism	Glutaraldehyde action
Bacterial spores	Low concentrations inhibit germination; high concentrations are sporicidal, probably as a consequence of strong interaction with outer cell layers
Mycobacteria	Action unknown, but probably involves mycobacterial cell wall
Other nonsporulating bacteria	Strong association with outer layers of gram-positive and gram-negative bacteria; cross-linking of amino groups in protein; inhibition of transport processes into cell
Fungi	Fungal cell wall appears to be a primary target site, with postulated interaction with chitin
Viruses	Actual mechanisms unknown, but involve protein-DNA cross-links and capsid changes
Protozoa	Mechanism of action not known

icroscopy. Glutaraldehyde has a broad spectrum of activity against bacteria and their spores, fungi, and viruses, and a considerable amount of information is now available about the ways whereby these different organisms are inactivated (Tables 2 and 3). Earlier reviews of its mechanisms of action have been published (179, 182, 374, 482).

The first reports in 1964 and 1965 (182) demonstrated that glutaraldehyde possessed high antimicrobial activity. Subsequently, research was undertaken to evaluate the nature of its bactericidal (339–344, 450) and sporicidal (180, 181, 507, 508) action. These bactericidal studies demonstrated (374) a strong binding of glutaraldehyde to outer layers of organisms such as *E. coli* and *Staphylococcus aureus* (179, 212, 339–341, 343, 344), inhibition of transport in gram-negative bacteria (179), inhibition of dehydrogenase activity (343, 344) and of periplasmic enzymes (179), prevention of lysostaphin-induced lysis in *S. aureus* (453) and of sodium lauryl sulfate-induced lysis in *E. coli* (340, 344), inhibition of spheroplast and protoplast lysis in hypotonic media (340, 344), and inhibition of RNA, DNA, and protein synthesis (320). Strong interaction of glutaraldehyde with lysine and other amino acids has been demonstrated (450).

Clearly, the mechanism of action of glutaraldehyde involves a strong association with the outer layers of bacterial cells, specifically with unprotonated amines on the cell surface, possibly representing the reactive sites (65). Such an effect could explain its inhibitory action on transport and on enzyme systems, where access of substrate to enzyme is prohibited. Partial or entire removal of the cell wall in hypertonic medium, leading to the production of spheroplasts or protoplasts and the subsequent prevention of lysis by glutaraldehyde when these forms are diluted in a hypotonic environment, suggests an additional effect on the inner membrane, a finding substantiated by the fact that the dialdehyde prevents the selective release of some membrane-bound enzymes of *Micrococcus lysodeikticus* (138). Glutaraldehyde is more active at alkaline than at acidic pHs. As the external pH is altered from acidic to alkaline, more reactive sites will be formed at the cell surface, leading to a more rapid bactericidal effect. The cross-links thus obtained mean that the cell is then unable to undertake most, if not all, of its essential functions. Glutaraldehyde is also mycobactericidal. Unfortunately, no critical studies have as yet been undertaken to evaluate the nature of this action (419).

The bacterial spore presents several sites at which interaction with glutaraldehyde is possible, although interaction with a particular site does not necessarily mean that this is associated with spore inactivation. *E. coli*, *S. aureus*, and vegetative cells of *Bacillus subtilis* bind more glutaraldehyde than do resting spores of *B. subtilis* (377, 378); uptake of glutaraldehyde is greater during germination and outgrowth than with mature spores but still lower than with vegetative cells. Low concentrations of the dialdehyde (0.1%) inhibit germination, whereas much higher concentrations (2%) are sporicidal. The aldehyde, at both acidic and alkaline pHs, interacts strongly with the outer spore layers (508, 509); this interaction reduces the release of dipicolinic acid (DPA) from heated spores and the lysis induced by mercaptoethanol (or thioglycolate)-peroxide combinations. Low concentrations of both acidic and alkaline glutaraldehyde increase the surface hydrophobicity of spores, again indicating an effect at the outermost regions of the cell. It has been observed by various authors (182, 374, 376, 380) that the greater sporicidal activity of glutaraldehyde at alkaline pH is not reflected by differences in uptake; however, uptake per se reflects binding and not necessarily penetration into the spore. It is conceivable that acidic glutaraldehyde interacts with and remains at the cell surface whereas alkaline glutaraldehyde penetrates more deeply into the spore. This contention is at odds with the hypothesis of Bruch (65), who envisaged the acidic form penetrating the coat and reacting with the cortex while the alkaline form attacked the coat, thereby destroying the ability of the spore to function solely as a result of this surface phenomenon. There is, as yet, no evidence to support this theory. Novel glutaraldehyde formulations based on acidic rather than alkaline glutaraldehyde, which benefit from the greater inherent stability of the aldehyde at lower pH, have been produced. The improved sporicidal activity claimed for these products may be obtained by agents that potentiate the activity of the dialdehyde (414, 421).

During sporulation, the cell eventually becomes less susceptible to glutaraldehyde (see "Intrinsic resistance of bacterial spores"). By contrast, germinating and outgrowing cells reacquire sensitivity. Germination may be defined as an irreversible process in which there is a change of an activated spore from a dormant to a metabolically active state within a short period. Glutaraldehyde exerts an early effect on the germination process. L-Alanine is considered to act by binding to a specific receptor on the spore coat, and once spores are triggered to germinate, they are committed irreversibly to losing their dormant properties (491). Glutaraldehyde at high concentrations inhibits the uptake of L-[^{14}C]alanine by *B. subtilis* spores, albeit by an unknown mechanism (379, 414). Glutaraldehyde-treated spores retain their refractivity, having the same appearance under the phase-contrast microscope as normal, untreated spores even when the spores are subsequently incubated in germination medium. Glutaraldehyde is normally used as a 2% solution to achieve a sporicidal effect (16, 316); low concentrations (<0.1%) prevent phase darkening of spores and also prevent the decrease in optical density associated with a late event in germination. By contrast, higher concentrations (0.1 to 1%) significantly reduce the uptake of L-alanine, possibly as a result of a sealing effect of the aldehyde on the cell surface. Mechanisms involved in the revival of glutaraldehyde-treated spores are discussed below (see "Intrinsic resistance of bacterial spores").

There are no recent studies of the mechanisms of fungicidal action of glutaraldehyde. Earlier work had suggested that the fungal cell wall was a major target site (179, 182, 352), especially the major wall component, chitin, which is analogous to the peptidoglycan found in bacterial cell walls.

Glutaraldehyde is a potent virucidal agent (143, 260). It reduces the activity of hepatitis B surface antigen (HBsAg) and especially hepatitis B core antigen ([HBcAg] in hepatitis B virus [HBV]) (3) and interacts with lysine residues on the surface of hepatitis A virus (HAV) (362). Low concentrations

(<0.1%) of alkaline glutaraldehyde are effective against purified poliovirus, whereas poliovirus RNA is highly resistant to aldehyde concentrations up to 1% at pH 7.2 and is only slowly inactivated at pH 8.3 (21). In other words, the complete poliovirus particle is much more sensitive than poliovirus RNA. In light of this, it has been inferred that glutaraldehyde-induced loss of infectivity is associated with capsid changes (21). Glutaraldehyde at the low concentrations of 0.05 and 0.005% interacts with the capsid proteins of poliovirus and echovirus, respectively; the differences in sensitivity probably reflect major structural variations in the two viruses (75).

Bacteriophages were recently studied to obtain information about mechanisms of virucidal action (298–304, 306, 307). Many glutaraldehyde-treated *P. aeruginosa* F116 phage particles had empty heads, implying that the phage genome had been ejected. The aldehyde was possibly bound to F116 double-stranded DNA but without affecting the molecule; glutaraldehyde also interacted with phage F116 proteins, which were postulated to be involved in the ejection of the nucleic acid. Concentrations of glutaraldehyde greater than 0.1 to 0.25% significantly affected the transduction of this phage; the transduction process was more sensitive to the aldehyde than was the phage itself. Glutaraldehyde and other aldehydes were tested for their ability to form protein-DNA cross-links in simian virus 40 (SV40); aldehydes (i.e., glyoxal, furfural, prionaldehyde, acetaldehyde, and benzylaldehyde) without detectable cross-linking ability had no effect on SV40 DNA synthesis, whereas acrolein, glutaraldehyde, and formaldehyde, which formed such cross-links (144, 271, 297), inhibited DNA synthesis (369).

Formaldehyde. Formaldehyde (methanal, CH_2O) is a monoaldehyde that exists as a freely water-soluble gas. Formaldehyde solution (formalin) is an aqueous solution containing *ca.* 34 to 38% (wt/wt) CH_2O with methanol to delay polymerization. Its clinical use is generally as a disinfectant and sterilant in liquid or in combination with low-temperature steam. Formaldehyde is bactericidal, sporicidal, and virucidal, but it works more slowly than glutaraldehyde (374, 482).

Formaldehyde is an extremely reactive chemical (374, 442) that interacts with protein (156, 157), DNA (155), and RNA (155) in vitro. It has long been considered to be sporicidal by virtue of its ability to penetrate into the interior of bacterial spores (500). The interaction with protein results from a combination with the primary amide as well as with the amino groups, although phenol groups bind little formaldehyde (155). It has been proposed that formaldehyde acts as a mutagenic agent (291) and as an alkylating agent by reaction with carboxyl, sulfhydryl, and hydroxyl groups (371). Formaldehyde also reacts extensively with nucleic acid (489) (e.g., the DNA of bacteriophage T2) (190). As pointed out above, it forms protein-DNA cross-links in SV40, thereby inhibiting DNA synthesis (369). Low concentrations of formaldehyde are sporostatic and inhibit germination (512). Formaldehyde alters HBsAg and HBcAg of HBV (3).

It is difficult to pinpoint accurately the mechanism(s) responsible for formaldehyde-induced microbial inactivation. Clearly, its interactive, and cross-linking properties must play a considerable role in this activity. Most of the other aldehydes (glutaraldehyde, glyoxyl, succinaldehyde, and *o*-phthalaldehyde [OPA]) that have sporicidal activity are dialdehydes (and of these, glyoxyl and succinaldehyde are weakly active). The distance between the two aldehyde groups in glutaraldehyde (and possibly in OPA) may be optimal for interaction of these-CHO groups in nucleic acids and especially in proteins and enzymes (428).

Formaldehyde-releasing agents. Several formaldehyde-releasing agents have been used in the treatment of peritonitis (226, 273). They include noxythiolin (oxymethylenethiourea), tauroline (a condensate of two molecules of the aminosulponic acid taurine with three molecules of formaldehyde), hexamine (hexamethylenetetramine, methenamine), the resins melamine and urea formaldehydes, and imidazolone derivatives such as dantoin. All of these agents are claimed to be microbicidal on account of the release of formaldehyde. However, because the antibacterial activity of taurolin is greater than that of free formaldehyde, the activity of taurolin is not entirely the result of formaldehyde action (247).

TABLE 4. Mechanisms of antimicrobial action of chlorhexidine

Type of microorganism	Chlorhexidine action
Bacterial spores	Not sporicidal but prevents development of spores; inhibits spore outgrowth but not germination
Mycobacteria	Mycobacteristatic (mechanism unknown) but not mycobactericidal
Other nonsporulating bacteria	Membrane-active agent, causing protoplast and spheroplast lysis; high concentrations cause precipitation of proteins and nucleic acids
Yeasts	Membrane-active agent, causing protoplast lysis and intracellular leakage; high concentrations cause intracellular coagulation
Viruses	Low activity against many viruses; lipid-enveloped viruses more sensitive than nonenveloped viruses; effect possibly on viral envelope, perhaps the lipid moieties
Protozoa	Recent studies against *A. castellanii* demonstrate membrane activity (leakage) toward trophozoites, less toward cysts

***o*-Phthalaldehyde.** OPA is a new type of disinfectant that is claimed to have potent bactericidal and sporicidal activity and has been suggested as a replacement for glutaraldehyde in endoscope disinfection (7). OPA is an aromatic compound with two aldehyde groups. To date, the mechanism of its antimicrobial action has been little studied, but preliminary evidence (526) suggests an action similar to that of glutaraldehyde. Further investigations are needed to corroborate this opinion.

Anilides

The anilides have been investigated primarily for use as antiseptics, but they are rarely used in the clinic. Triclocarban (TCC; 3,4,4′-triclorocarbanilide) is the most extensively studied in this series and is used mostly in consumer soaps and deodorants. TCC is particularly active against gram-positive bacteria but significantly less active against gram-negative bacteria and fungi (30) and lacks appreciable substantivity (persistency) for the skin (37). The anilides are thought to act by adsorbing to and destroying the semipermeable character of the cytoplasmic membrane, leading to cell death (194).

Biguanides

Chlorhexidine. Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in handwashing and oral products but also as a disinfectant and preservative. This is due in particular to its broad-spectrum efficacy, substantivity for the skin, and low irritation. Of note, irritability has been described and in many cases may be product specific (167, 403). Despite the advantages of chlorhexidine, its activity is pH dependent and is greatly reduced in the presence of organic matter (430). A considerable amount of research has been undertaken on the mechanism of the antimicrobial action of this important bisbiguanide (389) (Tables 2 and 4), although most of the attention has been devoted to the way in which it

inactivates nonsporulating bacteria (215, 428, 430, 431, 451). Nevertheless, sufficient data are now available to examine its sporostatic and mycobacteriostatic action, its effects on yeasts and protozoa, and its antiviral activity.

Chlorhexidine is a bactericidal agent (120, 215). Its interaction and uptake by bacteria were studied initially by Hugo et al. (222–224), who found that the uptake of chlorhexidine by *E. coli* and *S. aureus* was very rapid and depended on the chlorhexidine concentration and pH. More recently, by using [^{14}C]chlorhexidine gluconate, the uptake by bacteria (145) and yeasts (204) was shown to be extremely rapid, with a maximum effect occurring within 20 s. Damage to the outer cell layers takes place (139) but is insufficient to induce lysis or cell death. The agent then crosses the cell wall or outer membrane, presumably by passive diffusion, and subsequently attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. In yeasts, chlorhexidine "partitions" into the cell wall, plasma membrane, and cytoplasm of cells (205). Damage to the delicate semipermeable membrane is followed by leakage of intracellular constituents, which can be measured by appropriate techniques. Leakage is not per se responsible for cellular inactivation but is a consequence of cell death (445). High concentrations of chlorhexidine cause coagulation of intracellular constituents. As a result, the cytoplasm becomes congealed, with a consequent reduction in leakage (222–224, 290), so that there is a biphasic effect on membrane permeability. An initial high rate of leakage rises as the concentration of chlorhexidine increases, but leakage is reduced at higher biocide concentrations because of the coagulation of the cytosol.

Chlorhexidine was claimed by Harold et al. (199) to be an inhibitor of both membrane-bound and soluble ATPase as well as of net K^+ uptake in *Enterococcus faecalis*. However, only high biguanide concentrations inhibit membrane-bound ATPase (83), which suggests that the enzyme is not a primary target for chlorhexidine action. Although chlorhexidine collapses the membrane potential, it is membrane disruption rather than ATPase inactivation that is associated with its lethal effects (24, 272).

The effects of chlorhexidine on yeast cells are probably similar to those previously described for bacteria (204–207). Chlorhexidine has a biphasic effect on protoplast lysis, with reduced lysis at higher biguanide concentrations. Furthermore, in whole cells, the yeast cell wall may have some effect in limiting the uptake of the biguanide (208). The findings presented here and elsewhere (47, 136, 137, 527) demonstrate an effect on the fungal plasma membrane but with significant actions elsewhere in the cell (47). Increasing concentrations of chlorhexidine (up to 25 μg/ml) induce progressive lysis of *Saccharomyces cerevisiae* protoplasts, but higher biguanide concentrations result in reduced lysis (205).

Work to date suggests that chlorhexidine has a similar effect on the trophozoites of *Acanthameoba castellanii*, with the cysts being less sensitive (251–255). Furr (163) reviewed the effects of chlorhexidine and other biocides on *Acanthameoba* and showed that membrane damage in these protozoa is a significant factor in their inactivation.

Mycobacteria are generally highly resistant to chlorhexidine (419). Little is known about the uptake of chlorhexidine (and other antiseptics and disinfectants) by mycobacteria and on the biochemical changes that occur in the treated cells. Since the MICs for some mycobacteria are on the order of those for chlorhexidine-sensitive, gram-positive cocci (48), the inhibitory effects of chlorhexidine on mycobacteria may not be dissimilar to those on susceptible bacteria. *Mycobacterium avium-intracellulare* is considerably more resistant than other mycobacteria (48).

Chlorhexidine is not sporicidal (discussed in "Mechanisms of resistance"). Even high concentrations of the bisbiguanide do not affect the viability of *Bacillus* spores at ambient temperatures (473, 474), although a marked sporicidal effect is achieved at elevated temperatures (475). Presumably, sufficient changes occur in the spore structure to permit an increased uptake of the biguanide, although this has yet to be shown experimentally. Little is known about the uptake of chlorhexidine by bacterial spores, although coatless forms take up more of the compound than do "normal" spores (474).

Chlorhexidine has little effect on the germination of bacterial spores (414, 422, 432, 447) but inhibits outgrowth (447). The reason for its lack of effect on the former process but its significant activity against the latter is unclear. It could, however, be reflected in the relative uptake of chlorhexidine, since germinating cells take up much less of the bisbiguanide than do outgrowing forms (474). Binding sites could thus be reduced in number or masked in germinating cells.

The antiviral activity of chlorhexidine is variable. Studies with different types of bacteriophages have shown that chlorhexidine has no effect on MS2 or K coliphages (300). High concentrations also failed to inactivate *Pseudomonas aeruginosa* phage F116 and had no effect on phage DNA within the capsid or on phage proteins (301); the transduction process was more sensitive to chlorhexidine and other biocides than was the phage itself. This substantiated an earlier finding (306) that chlorhexidine bound poorly to F116 particles. Chlorhexidine is not always considered a particularly effective antiviral agent, and its activity is restricted to the lipid-enveloped viruses (361). Chlorhexidine does not inactivate nonenveloped viruses such as rotavirus (485), HAV (315), or poliovirus (34). Its activity was found by Ranganathan (389) to be restricted to the nucleic acid core or the outer coat, although it is likely that the latter would be a more important target site.

Alexidine. Alexidine differs chemically from chlorhexidine in possessing ethylhexyl end groups. Alexidine is more rapidly bactericidal and produces a significantly faster alteration in bactericidal permeability (79, 80). Studies with mixed-lipid and pure phospholipid vesicles demonstrate that, unlike chlorhexidine, alexidine produces lipid phase separation and domain formation (Table 2). It has been proposed (80) that the nature of the ethylhexyl end group in alexidine, as opposed to the chlorophenol one in chlorhexidine, might influence the ability of a biguanide to produce lipid domains in the cytoplasmic membrane.

Polymeric biguanides. Vantocil is a heterodisperse mixture of polyhexamethylene biguanides (PHMB) with a molecular weight of approximately 3,000. Polymeric biguanides have found use as general disinfecting agents in the food industry and, very successfully, for the disinfection of swimming pools. Vantocil is active against gram-positive and gram-negative bacteria, although *P. aeruginosa* and *Proteus vulgaris* are less sensitive. Vantocil is not sporicidal. PHMB is a membrane-active agent that also impairs the integrity of the outer membrane of gram-negative bacteria, although the membrane may also act as a permeability barrier (64, 172). Activity of PHMB increases on a weight basis with increasing levels of polymerization, which has been linked to enhanced inner membrane perturbation (173, 174).

Unlike chlorhexidine but similar to alexidine (Table 2), PHMB causes domain formation of the acidic phospholipids of the cytoplasmic membrane (61–64, 172, 173, 227). Permeability changes ensue, and there is believed to be an altered function of some membrane-associated enzymes. The proposed sequence of events during its interaction with the cell envelope of *E. coli* is as follows: (i) there is rapid attraction of

PHMB toward the negatively charged bacterial cell surface, with strong and specific adsorption to phosphate-containing compounds; (ii) the integrity of the outer membrane is impaired, and PHMB is attracted to the inner membrane; (iii) binding of PHMB to phospholipids occurs, with an increase in inner membrane permeability (K^+ loss) accompanied by bacteriostasis; and (iv) complete loss of membrane function follows, with precipitation of intracellular constituents and a bactericidal effect.

Diamidines

The diamidines are characterized chemically as described in Table 1. The isethionate salts of two compounds, propamidine (4,4-diaminodiphenoxypropane) and dibromopropamidine (2,2-dibromo-4,4-diamidinodiphenoxypropane), have been used as antibacterial agents. Their antibacterial properties and uses were reviewed by Hugo (213) and Hugo and Russell (226). Clinically, diamidines are used for the topical treatment of wounds.

The exact mechanism of action of diamidines is unknown, but they have been shown to inhibit oxygen uptake and induce leakage of amino acids (Table 2), as would be expected if they are considered as cationic surface-active agents. Damage to the cell surface of *P. aeruginosa* and *Enterobacter cloacae* has been described (400).

Halogen-Releasing Agents

Chlorine- and iodine-based compounds are the most significant microbicidal halogens used in the clinic and have been traditionally used for both antiseptic and disinfectant purposes.

Chlorine-releasing agents. Excellent reviews that deal with the chemical, physical, and microbiological properties of chlorine-releasing agents (CRAs) are available (42, 130). The most important types of CRAs are sodium hypochlorite, chlorine dioxide, and the *N*-chloro compounds such as sodium dichloroisocyanurate (NaDCC), with chloramine-T being used to some extent. Sodium hypochlorite solutions are widely used for hard-surface disinfection (household bleach) and can be used for disinfecting spillages of blood containing human immunodeficiency virus or HBV. NaDCC can also be used for this purpose and has the advantages of providing a higher concentration of available chlorine and being less susceptible to inactivation by organic matter. In water, sodium hypochlorite ionizes to produce Na^+ and the hypochlorite ion, OCl^-, which establishes an equilibrium with hypochlorous acid, HOCl (42). Between pH 4 and 7, chlorine exists predominantly as HClO, the active moiety, whereas above pH9, OCl^- predominates. Although CRAs have been predominantly used as hard-surface disinfectants, novel acidified sodium chlorite (a two-component system of sodium chlorite and mandelic acid) has been described as an effective antiseptic (248).

Surprisingly, despite being widely studied, the actual mechanism of action of CRAs is not fully known (Table 2). CRAs are highly active oxidizing agents and thereby destroy the cellular activity of proteins (42); potentiation of oxidation may occur at low pH, where the activity of CRAs is maximal, although increased penetration of outer cell layers may be achieved with CRAs in the unionized state. Hypochlorous acid has long been considered the active moiety responsible for bacterial inactivation by CRAs, the OCl^- ion having a minute effect compared to undissolved HOCl (130). This correlates with the observation that CRA activity is greatest when the percentage of undissolved HOCl is highest. This concept applies to hypochlorites, NaDCC, and chloramine-T.

Deleterious effects of CRAs on bacterial DNA that involve the formation of chlorinated derivatives of nucleotide bases have been described (115, 128, 477). Hypochlorous acid has also been found to disrupt oxidative phosphorylation (26) and other membrane-associated activity (70). In a particularly interesting paper, McKenna and Davies (321) described the inhibition of bacterial growth by hypochlorous acid. At 50 μM (2.6 ppm), HOCl completely inhibited the growth of *E. coli* within 5 min, and DNA synthesis was inhibited by 96% but protein synthesis was inhibited by only 10 to 30%. Because concentrations below 5 mM (260 ppm) did not induce bacterial membrane disruption or extensive protein degradation, it was inferred that DNA synthesis was the sensitive target. In contrast, chlorine dioxide inhibited bacterial protein synthesis (33).

CRAs at higher concentrations are sporicidal (44, 421, 431); this depends on the pH and concentration of available chlorine (408, 412). During treatment, the spores lose refractivity, the spore coat separates from the cortex, and lysis occurs (268). In addition, a number of studies have concluded that CRA-treated spores exhibit increased permeability of the spore coat (131, 268, 412).

CRAs also possess virucidal activity (34, 46, 116, 315, 394, 407, 467, 485, 486). Olivieri et al. (359) showed that chlorine inactivated naked f2 RNA at the same rate as RNA in intact phage, whereas f2 capsid proteins could still adsorb to the host. Taylor and Butler (504) found that the RNA of poliovirus type 1 was degraded into fragments by chlorine but that poliovirus inactivation preceded any severe morphological changes. By contrast, Floyd et al. (149) and O'Brien and Newman (357) demonstrated that the capsid of poliovirus type 1 was broken down. Clearly, further studies are needed to explain the antiviral action of CRAs.

Iodine and iodophors. Although less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal (184). Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they are associated with irritation and excessive staining. In addition, aqueous solutions are generally unstable; in solution, at least seven iodine species are present in a complex equilibrium, with molecular iodine (I_2) being primarily responsible for antimictrobial efficacy (184). These problems were overcome by the development of iodophors ("iodine carriers" or "iodine-releasing agents"); the most widely used are povidone-iodine and poloxamer-iodine in both antiseptics and disinfectants. Iodophors are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active "free" iodine (184). Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures (454).

Similar to chlorine, the antimicrobial action of iodine is rapid, even at low concentrations, but the exact mode of action is unknown. Iodine rapidly penetrates into microorganisms (76) and attacks key groups of proteins (in particular the free-sulfur amino acids cysteine and methionine [184, 267]), nucleotides, and fatty acids (15, 184), which culminates in cell death (184). Less is known about the antiviral action of iodine, but nonlipid viruses and parvoviruses are less sensitive than lipid enveloped viruses (384). Similarly to bacteria, it is likely that iodine attacks the surface proteins of enveloped viruses, but they may also destabilize membrane fatty acids by reacting with unsaturated carbon bonds (486).

Silver Compounds

In one form or another, silver and its compounds have long been used as antimicrobial agents (55, 443). The most important silver compound currently in use is silver sulfadiazine

(AgSD), although silver metal, silver acetate, silver nitrate, and silver protein, all of which have antimicrobial properties, are listed in *Martindale, The Extra Pharmacopoeia* (312). In recent years, silver compounds have been used to prevent the infection of burns and some eye infections and to destroy warts.

Silver nitrate. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfydryl, -SH) groups (32, 49, 161, 164), although other target sites remain a possibility (397, 509). Liau et al (287) demonstrated that amino acids such as cysteine and other compounds such as sodium thioglycolate containing thiol groups neutralized the activity of silver nitrate against *P. aeruginosa*. By contrast, amino acids containing disulfide (SS) bonds, non-sulfur-containing amino acids, and sulfur-containing compounds such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfite, and sodium thiosulfate were all unable to neutralize Ag^+ activity. These and other findings imply that interaction of Ag^+ with thiol groups in enzymes and proteins plays an essential role in bacterial inactivation, although other cellular components may be involved. Hydrogen bonding, the effects of hydrogen bond-breaking agents, and the specificity of Ag^+ for thiol groups were discussed in greater detail by Russell and Hugo (443) (Table 2). Virucidal properties might also be explained by binding to -SH groups (510).

Lukens (292) proposed that silver salts and other heavy metals such as copper act by binding to key functional groups of fungal enzymes. Ag^+ causes the release of K^+ ions from microorganisms; the microbial plasma or cytoplasmic membrane, with which is associated many important enzymes, is an important target site for Ag^+ activity (161, 329, 392, 470).

In addition to its effects on enzymes, Ag^+ produces other changes in microorganisms. Silver nitrate causes marked inhibition of growth of *Cryptococcus neoformans* and is deposited in the vacuole and cell wall as granules (60). Ag^+ inhibits cell division and damages the cell envelope and contents of *P. aeruginosa* (398). Bacterial cells increase in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibit structural abnormalities, although without any blebs (protuberances) (398). Finally, the Ag^+ ion interacts with nucleic acids (543); it interacts preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of its lethal action is unclear (231, 387, 510, 547).

Silver sulfadiazine. AgSD is essentially a combination of two antibacterial agents, Ag^+ and sulfadiazine (SD). The question whether the antibacterial effect of AgSD arises predominantly from only one of the compounds or via a synergistic interaction has been posed repeatedly. AgSD has a broad spectrum of activity and, unlike silver nitrate, produces surface and membrane blebs in susceptible (but not resistant) bacteria (96). AgSD binds to cell components, including DNA (332, 404). Based on a chemical analysis, Fox (153) proposed a polymeric structure of AgSD composed of six silver atoms bonding to six SD molecules by linkage of the silver atoms to the nitrogens of the SD pyrimidine ring. Bacterial inhibition would then presumably be achieved when silver binds to sufficient base pairs in the DNA helix, thereby inhibiting transcription. Similarly, its antiphage properties have been ascribed to the fact that AgSD binds to phage DNA (154, 388). Clearly, the precise mechanism of action of AgSD has yet to be solved.

Peroxygens

Hydrogen peroxide. Hydrogen peroxide (H_2O_2) is a widely used biocide for disinfection, sterilization, and antisepsis. It is a clear, colorless liquid that is commercially available in a variety of concentrations ranging from 3 to 90%. H_2O_2 is considered environmentally friendly, because it can rapidly degrade into the innocuous products water and oxygen. Although pure solutions are generally stable, most contain stabilizers to prevent decomposition. H_2O_2 demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores (38). In general, greater activity is seen against gram-positive than gram-negative bacteria; however, the presence of catalase or other peroxidases in these organisms can increase tolerance in the presence of lower concentrations. Higher concentrations of H_2O_2 (10 to 30%) and longer contact times are required for sporicidal activity (416), although this activity is significantly increased in the gaseous phase. H_2O_2 acts as an oxidant by producing hydroxyl free radicals ($^{\bullet}OH$) which attack essential cell components, including lipids, proteins, and DNA. It has been proposed that exposed sulfhydryl groups and double bonds are particularly targeted (38).

Peracetic acid. Peracetic acid (PAA) (CH_3COOOH) is considered a more potent biocide than hydrogen peroxide, being sporicidal, bactericidal, virucidal, and fungicidal at low concentrations (<0.3%) (38). PAA also decomposes to safe by-products (acetic acid and oxygen) but has the added advantages of being free from decomposition by peroxidases, unlike H_2O_2, and remaining active in the presence of organic loads (283, 308). Its main application is as a low-temperature liquid sterilant for medical devices, flexible scopes, and hemodialyzers, but it is also used as an environmental surface sterilant (100, 308).

Similar to H_2O_2, PAA probably denatures proteins and enzymes and increases cell wall permeability by disrupting sulfhydryl (-SH) and sulfur (S-S) bonds (22, 38).

Phenols

Phenolic-type antimicrobial agents have long been used for their antiseptic, disinfectant, or preservative properties, depending on the compound. It has been known for many years (215) that, although they have often been referred to as "general protoplasmic poisons," they have membrane-active properties which also contribute to their overall activity (120) (Table 2).

Phenol induces progressive leakage of intracellular constituents, including the release of K^+, the first index of membrane damage (273), and of radioactivity from ^{14}C-labeled *E. coli* (242, 265). Pulvertaft and Lumb (386) demonstrated that low concentrations of phenols (0.032%, 320 μg/ml) and other (nonphenolic) agents lysed rapidly growing cultures of *E. coli*, staphylococci, and streptococci and concluded that autolytic enzymes were not involved. Srivastava and Thompson (487, 488) proposed that phenol acts only at the point of separation of pairs of daughter cells, with young bacterial cells being more sensitive than older cells to phenol.

Hugo and Bloomfield (216, 217) showed with the chlorinated bis-phenol fenticlor that there was a close relationship between bactericidal activity and leakage of 260-nm-absorbing material (leakage being induced only by bactericidal concentrations). Fentichlor affected the metabolic activities of *S. aureus* and *E. coli* (217) and produced a selective increase in permeability to protons with a consequent dissipation of the proton motive force (PMF) and an uncoupling of oxidative phosphorylation (41). Chlorocresol has a similar action (124). Coagulation of cytoplasmic constituents at higher phenol concentrations, which causes irreversible cellular damage, has been described by Hugo (215).

The phenolics possess antifungal and antiviral properties. Their antifungal action probably involves damage to the plas-

ma membrane (436), resulting in leakage of intracellular constituents. Phenol does not affect the transduction of *P. aeruginosa* PAO by bacteriophage F116 (301), has no effect on phage DNA within the capsid, and has little effect on several of the phage band proteins unless treatments of 20 min or longer are used (303, 304).

Bis-Phenols

The bis-phenols are hydroxy-halogenated derivatives of two phenolic groups connected by various bridges (191, 446). In general, they exhibit broad-spectrum efficacy but have little activity against *P. aeruginosa* and molds and are sporostatic toward bacterial spores. Triclosan and hexachlorophane are the most widely used biocides in this group, especially in antiseptic soaps and hand rinses. Both compounds have been shown to have cumulative and persistent effects on the skin (313).

Triclosan. Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether; Irgasan DP 300) exhibits particular activity against gram-positive bacteria (469, 521). Its efficacy against gram-negative bacteria and yeasts can be significantly enhanced by formulation effects. For example, triclosan in combination with EDTA caused increased permeability of the outer membrane (282). Reports have also suggested that in addition to its antibacterial properties, triclosan may have anti-inflammatory activity (25, 522). The specific mode of action of triclosan is unknown, but it has been suggested that the primary effects are on the cytoplasmic membrane. In studies with *E. coli*, triclosan at subinhibitory concentrations inhibited the uptake of essential nutrients, while higher, bactericidal concentrations resulted in the rapid release of cellular components and cell death (393). Studies with a divalent-ion-dependent *E. coli* triclosan mutant for which the triclosan MIC was 10-fold greater than that for a wild-type strain showed no significant differences in total envelope protein profiles but did show significant differences in envelope fatty acids (370). Specifically, a prominent 14:1 fatty acid was absent in the resistant strain, and there were minor differences in other fatty acid species. It was proposed that divalent ions and fatty acids may adsorb and limit the permeability of triclosan to its site of action (370). Minor changes in fatty acid profiles were recently found in both *E. coli* and *S. aureus* strains for which the triclosan MICs were elevated; however, the MBCs were not affected, suggesting, as for other phenols, that the cumulative effects on multiple targets contribute to the bactericidal activity (318, 319).

Hexachlorophene. Hexachlorophene (hexachlorophane; 2,2′-dihydroxy-3,5,6,3′,5′,6′-hexachlorodiphenylmethane) is another bis-phenol whose mode of action has been extensively studied. The primary action of hexachlorophene, based on studies with *Bacillus megatherium*, is to inhibit the membrane-bound part of the electron transport chain, and the other effects noted above are secondary ones that occur only at high concentrations (92, 158, 241, 481). It induces leakage, causes protoplast lysis, and inhibits respiration. The threshold concentration for the bactericidal activity of hexachlorphene is 10 μg/ml (dry weight), but peak leakage occurs at concentrations higher than 50 μg/ml and cytological changes occur above 30 μg/ml. Furthermore, hexachlorophene is bactericidal at 0°C despite causing little leakage at this temperature. Despite the broad-spectrum efficacy of hexachlorophene, concerns about toxicity (256), in particular in neonates, have meant that its use in antiseptic products has been limited.

Halophenols

Chloroxylenol (4-chloro-3,5-dimethylphenol; *p*-chloro-*m*-xylenol) is the key halophenol used in antiseptic or disinfectant formulations (66). Chloroxylenol is bactericidal, but *P. aeruginosa* and many molds are highly resistant (66, 432). Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it would be expected to have an effect on microbial membranes.

Quaternary Ammonium Compounds

Surface-active agents (surfactants) have two regions in their molecular structures, one a hydrocarbon, water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic or polar) group. Depending on the basis of the charge or absence of ionization of the hydrophilic group, surfactants are classified into cationic, anionic, nonionic, and ampholytic (amphoteric) compounds. Of these, the cationic agents, as exemplified by quaternary ammonium compounds (QACs), are the most useful antiseptics and disinfectants (160). They are sometimes known as cationic detergents. QACs have been used for a variety of clinical purposes (e.g., preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of noncritical surfaces). In addition to having antimicrobial properties, QACs are also excellent for hard-surface cleaning and deodorization.

It has been known for many years that QACs are membrane-active agents (221) (Table 2) (i.e., with a target site predominantly at the cytoplasmic (inner) membrane in bacteria or the plasma membrane in yeasts) (215). Salton (460) proposed the following sequence of events with microorganisms exposed to cationic agents: (i) adsorption and penetration of the agent into the cell wall; (ii) reaction with the cytoplasmic membrane (lipid or protein) followed by membrane disorganization; (iii) leakage of intracellular low-molecular-weight material; (iv) degradation of proteins and nucleic acids; and (v) wall lysis caused by autolytic enzymes. There is thus a loss of structural organization and integrity of the cytoplasmic membrane in bacteria, together with other damaging effects to the bacterial cell (120).

Useful information about the selectivity of membrane action can be obtained by studying the effects of biocides on protoplasts and spheroplasts suspended in various solutes. QACs cause lysis of spheroplasts and protoplasts suspended in sucrose (107, 215, 243, 428). The cationic agents react with phospholipid components in the cytoplasmic membrane (69), thereby producing membrane distortion and protoplast lysis under osmotic stress. Isolated membranes do not undergo disaggregation on exposure to QACs, because the membrane distortion is not sufficiently drastic. The non-QAC agents TCC and trichlorosalicylanide have specific effects: TCC induces protoplast lysis in ammonium chloride by increasing Cl^- permeability, whereas trichlorosalicylanide induces lysis in ammonium nitrate by increasing NO_3^- permeability (428). In contrast, QACs (and chlorhexidine) induce lysis of protoplasts or spheroplasts suspended in various solutes because they effect generalized, rather than specific, membrane damage.

The bacterial cytoplasmic membrane provides the mechanism whereby metabolism is linked to solute transport, flagellar movement, and the generation of ATP. Protons are extruded to the exterior of the bacterial cell during metabolism. The combined potential (concentration or osmotic effect of the proton and its electropositivity) is the PMF, which drives these ancillary activities (428). The QAC cetrimide was found (121) to have an effect on the PMF in *S. aureus*. At its bacteriostatic concentration, cetrimide caused the discharge of the pH component of the PMF and also produced the maximum amount of 260-nm-absorbing material.

QACs are also believed to damage the outer membrane of gram-negative bacteria, thereby promoting their own uptake. This aspect of QACs is considered below (see "Intrinsic resistance of gram-negative bacteria").

The QAC cetylpyridium chloride (CPC) induces the leakage of K^+ and pentose material from the yeast *S. cerevisiae* and induces protoplast lysis as well as interacting with crude cell sap (205). Unlike chlorhexidine, however, no biphasic effect on protoplast lysis was observed. The initial toxic effect of QACs on yeast cells is a disorganization of the plasma membranes, with organized lipid structures in the membranes (and in lipid bilayers) being disrupted.

QACs are sporostatic; they inhibit the outgrowth of spores (the development of a vegetative cell from a germinated spore) but not the actual germination processes (development from dormancy to a metabolically active state), albeit by an unknown mechanism (414). Likewise, the QACs are not mycobactericidal but have a mycobacteriostatic action, although the actual effects on mycobacteria have been little studied (419).

The QACs have an effect on lipid, enveloped (including human immunodeficiency virus and HBV) but not nonenveloped viruses (394, 485, 486). QAC-based products induced disintegration and morphological changes of human HBV, resulting in loss of infectivity (382). In studies with different phages (298–301, 303–305, 307), CPC significantly inhibited transduction by bacteriophage F116 and inactivated the phage particles. Furthermore, CPC altered the protein bands of F116 but did not affect the phage DNA within the capsid.

Vapor-Phase Sterilants

Many heat-sensitive medical devices and surgical supplies can be effectively sterilized by liquid sterilants (in particular glutaraldehyde, PAA, and hydrogen peroxide) or by vapor-phase sterilization systems (Table 1). The most widely used active agents in these "cold" systems are ethylene oxide, formaldehyde and, more recently developed, hydrogen peroxide and PAA. Ethylene oxide and formaldehyde are both broad-spectrum alkylating agents. However, their activity is dependent on active concentration, temperature, duration of exposure, and relative humidity (87). As alkylating agents, they attack proteins, nucleic acids, and other organic compounds; both are particularly reactive with sulfhydryl and other enzyme-reactive groups. Ethylene oxide gas has the disadvantages of being mutagenic and explosive but is not generally harsh on sensitive equipment, and toxic residuals from the sterilization procedure can be routinely eliminated by correct aeration. Formaldehyde gas is similar and has the added advantage of being nonexplosive but is not widely used in health care. Vapor-phase hydrogen peroxide and PAA are considered more active (as oxidants) at lower concentrations than in the liquid form (334). Both active agents are used in combination with gas plasma in low-temperature sterilization systems (314). Their main advantages over other vapor-phase systems include low toxicity, rapid action, and activity at lower temperature; the disadvantages include limited penetrability and applications.

MECHANISMS OF RESISTANCE

Introduction

As stated above, different types of microorganisms vary in their response to antiseptics and disinfectants. This is hardly surprising in view of their different cellular structure, composition, and physiology. Traditionally, microbial susceptibility to antiseptics and disinfectants has been classified based on these differences; with recent work, this classification can be further extended (Fig. 1). Because different types of organisms react differently, it is convenient to consider bacteria, fungi, viruses, protozoa, and prions separately.

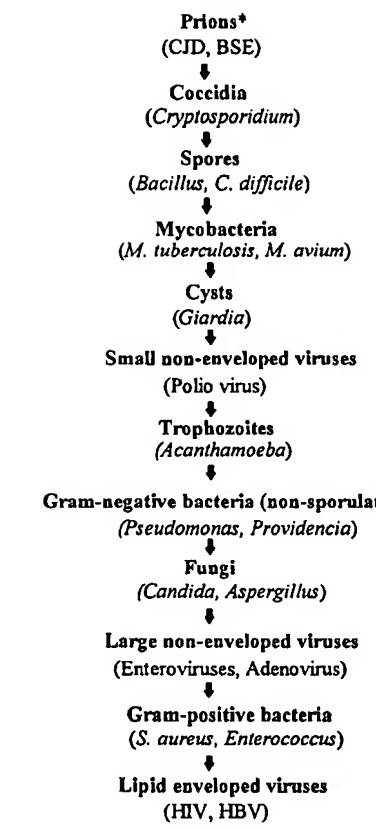


FIG. 1. Descending order of resistance to antiseptics and disinfectants. The asterisk indicates that the conclusions are not yet universally agreed upon.

Bacterial Resistance to Antiseptics and Disinfectants

In recent years, considerable progress has been made in understanding more fully the responses of different types of bacteria (mycobacteria, nonsporulating bacteria, and bacterial spores) to antibacterial agents (43, 84, 414, 415, 419, 422, 496). As a result, resistance can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids (self-replicating, extrachromosomal DNA) or transposons (chromosomal or plasmid integrating, transmissible DNA cassettes). Intrinsic resistance is demonstrated by gram-negative bacteria, bacterial spores, mycobacteria, and, under certain conditions, staphylococci (Table 5). Acquired, plasmid-mediated resistance is most widely associated with mercury compounds and other metallic salts. In recent years, acquired resistance to certain other types of biocides has been observed, notably in staphylococci.

Intrinsic Bacterial Resistance Mechanisms

For an antiseptic or disinfectant molecule to reach its target site, the outer layers of a cell must be crossed. The nature and composition of these layers depend on the organism type and may act as a permeability barrier, in which there may be a reduced uptake (422, 428). Alternatively but less commonly, constitutively synthesized enzymes may bring about degradation of a compound (43, 214, 358). Intrinsic (innate) resistance

TABLE 5. Intrinsic resistance mechanisms in bacteria to antiseptics and disinfectants

Type of resistance	Example(s)	Mechanism of resistance
Impermeability		
Gram-negative bacteria	QACs, triclosan, diamines	Barrier presented by outer membrane may prevent uptake of antiseptic or disinfectant; glycocalyx may also be involved
Mycobacteria	Chlorhexidine, QACs Glutaraldehyde	Waxy cell wall prevents adequate biocide entry Reason for high resistance of some strains of *M. chelonae*(?)
Bacterial spores	Chlorhexidine, QACs, phenolics	Spore coat(s) and cortex present a barrier to entry of antiseptics and disinfectants
Gram-positive bacteria	Chlorhexidine	Glycocalyx/mucoexopolysaccaride may be associated with reduced diffusion of antiseptic
Inactivation (chromosomally mediated)	Chlorohexidine	Breakdown of chlorhexidine molecule may be responsible for resistance

is thus a natural, chromosomally controlled property of a bacterial cell that enables it to circumvent the action of an antiseptic or disinfectant. Gram-negative bacteria tend to be more resistant than gram-positive organisms, such as staphylococci (Table 6).

Intrinsic resistance of bacterial spores. Bacterial spores of the genera *Bacillus* and *Clostridium* have been widely studied and are invariably the most resistant of all types of bacteria to antiseptics and disinfectants (43, 46, 150, 414, 418, 420, 422, 423, 457). Although *Bacillus* species are generally not pathogenic, their spores are widely used as indicators of efficient sterilization. *Clostridium* species are significant pathogens; for example, *C. difficile* is the most common cause of hospital-acquired diarrhea (478). Many biocides are bactericidal or bacteristatic at low concentrations for nonsporulating bacteria, including the vegetative cells of *Bacillus* and *Clostridium* species, but high concentrations may be necessary to achieve a sporicidal effect (e.g., for glutaraldehyde and CRAs). By contrast, even high concentrations of alcohol, phenolics, QACs, and chlorhexidine lack a sporicidal effect, although this may be achieved when these compounds are used at elevated temperatures (475).

A typical spore has a complex structure (29, 151). In brief, the germ cell (protoplast or core) and germ cell wall are surrounded by the cortex, outside which are the inner and outer spore coats. A thin exosporium may be present in the spores of some species but may surround just one spore coat. RNA, DNA, and DPA, as well as most of the calcium, potassium, manganese, and phosphorus, are present in the spore protoplast. Also present are large amounts of low-molecular-weight basic proteins (small acid-soluble spore proteins [SASPs]), which are rapidly degraded during germination. The cortex consists largely of peptidoglycan, including a spore-specific muramic lactam. The spore coats comprise a major portion of the spore. These structures consist largely of protein, with an alkali-soluble fraction made up of acidic polypeptides being found in the inner coat and an alkali-resistant fraction associated with the presence of disulfide-rich bonds being found in the outer coat. These aspects, especially the roles of the coat(s) and cortex, are all relevant to the mechanism(s) of resistance presented by bacterial spores to antiseptics and disinfectants.

Several techniques are available for studying mechanisms of spore resistance (428). They include removing the spore coat and cortex by using a "step-down" technique to achieve a highly synchronous sporulation (so that cellular changes can be accurately monitored), employing spore mutants that do not sporulate beyond genetically determined stages in sporulation, adding an antiseptic or disinfectant at the commencement of sporulation and determining how far the process can proceed, and examining the role of SASPs. Such procedures have helped provide a considerable amount of useful information. Sporulation itself is a process in which a vegetative cell develops into a spore and involves seven stages (designated 0 to VII). During this process, the vegetative cell (stage 0) undergoes a series of morphological changes that culminate in the release of a mature spore (stage VII). Stages IV (cortex development) to VII are the most important in the development of resistance to biocides.

Resistance to antiseptics and disinfectants develops during sporulation and may be an early, intermediate, or (very) late event (103, 375, 378, 429, 474). Useful markers for monitoring the development of resistance are toluene (resistance to which is an early event), heat (intermediate), and lysozyme (late) (236, 237). Studies with a wild-type *B. subtilis* strain, 168, and its Spo^- mutants have helped determine the stages at which resistance develops (262, 375, 474). From these studies (Fig. 2), the order of development of resistance was toluene (marker), formaldehyde, sodium lauryl sulfate, phenol, and phenylmercuric nitrate; *m*-cresol, chlorocresol, chlorhexidine gluconate, cetylpyridinium chloride, and mercuric chloride; and moist heat (marker), sodium dichloroisocyanurate, sodium hypochlorite, lysozyme (marker), and glutaraldehyde. The association of the onset of resistance to a particular antiseptic or disinfectant with a particular stage(s) in spore development is thereby demonstrated.

Spore coat-less forms, produced by treatment of spores un-

TABLE 6. MIC of some antiseptics and disinfectants against gram-positive and gram-negative bacteria[a]

Chemical agent	MIC (μg/ml) for:		
	S. aureus[b]	*E. coli*	*P. aeruginosa*
Benzalkonium chloride	0.5	50	250
Benzethonium chloride	0.5	32	250
Cetrimide	4	16	64–128
Chlorhexidine	0.5–1	1	5–60
Hexachlorophene	0.5	12.5	250
Phenol	2,000	2,000	2,000
o-Phenylphenol	100	500	1,000
Propamine isethionate	2	64	256
Dibromopropamidine isethionate	1	4	32
Triclosan	0.1	5	>300

[a] Based on references 226 and 440.
[b] MICs of cationic agents for some MRSA strains may be higher (see Table 10).

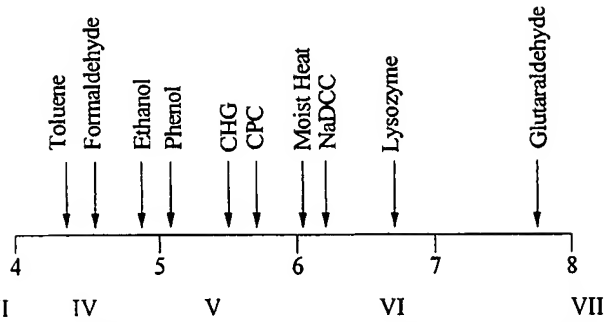


FIG. 2. Development of resistance of *Bacillus subtilis* during sporulation. Roman numerals indicate the sporulation stage from III (engulfment of the forespore) to VII (release of the mature spore). Arabic numbers indicate the time (hours) following the onset of sporulation and the approximate times at which resistance develops against biocides (262). CHG, chlorhexidine; CPC, cetylpyridinium chloride; NaDCC, sodium dichloroisocyanurate.

der alkaline conditions with urea plus dithiothreitol plus sodium lauryl sulfate (UDS), have also been of value in estimating the role of the coats in limiting the access of antiseptics and disinfectants to their target sites. However, Bloomfield and Arthur (44, 45) and Bloomfield (43) showed that this treatment also removes a certain amount of cortex and that the amount of cortex remaining can be further reduced by the subsequent use of lysozyme. These findings demonstrate that the spore coats have an undoubted role in conferring resistance but that the cortex also is an important barrier since (UDS plus lysozyme)-treated spores are much more sensitive to chlorine- and iodine-releasing agents than are UDS-exposed spores.

The initial development and maturity of the cortex are implicated in the development of resistance to phenolics. Likewise, it is now clear that cortex development is at least partially responsible for resistance to chlorhexidine and QACs; this resistance is enhanced in developing spores by the initiation of spore coat synthesis (262). The effect of various concentrations of chlorhexidine, sublethal to vegetative bacteria, on the development of spores of *B. subtilis* 168 MB_2 were investigated by Knott and Russell (261). They found that chlorhexidine affected spore development; as concentrations of the biguanide increased, spore index values (the percentage of cells forming spores) decreased and sensitivity to both heat and toluene increased. By contrast, the control (untreated) culture was highly resistant to both of these agents and had a high spore index value, indicative of high levels of mature spores. The slightly increased resistance to toluene compared to resistance to heat was not surprising, since cells must reach stages V to VI (synthesis of spore coats and maturation) to attain heat resistance but only stage III (forespore engulfment) to attain toluene resistance (Fig. 2); in other words, if sporulation is inhibited by chlorhexidine, more cells are likely to reach stage III than the later stages. While less definitive than the earlier approaches, these procedures provide further evidence of the involvement of the cortex and coats in chlorhexidine resistance.

Development of resistance during sporulation to formaldehyde was an early event but depended to some extent on the concentration (1 to 5% [vol/vol]) of formaldehyde used. This appears to be at odds with the extremely late development of resistance to the dialdehyde, glutaraldehyde. Since glutaraldehyde and the monoaldehyde, formaldehyde, contain an aldehyde group(s) and are alkylating agents, it would be plausible to assume that they would have a similar mode of sporicidal action, even though the dialdehyde is a more powerful alkylating agent. If this were true, it could also be assumed that spores would exhibit the same resistance mechanisms for these disinfectants. In aqueous solution, formaldehyde forms a glycol in equilibrium (512, 524); thus, formaldehyde could well be acting poorly as an alcohol-type disinfectant rather than as an aldehyde (327). Alkaline glutaraldehyde does not readily form glycols in aqueous solution (178). Resistance to formaldehyde may be linked to cortex maturation, and resistance to glutaraldehyde may be linked to coat formation (262).

Setlow and his coworkers (472) demonstrated that α/β-type SASPs coat the DNA in wild-type spores of *B. subtilis*, thereby protecting it from attack by enzymes and antimicrobial agents. Spores ($\alpha^-\beta^-$) lacking these α/β-type SASPs are significantly more sensitive to hydrogen peroxide (471) and hypochlorite (456). Thus, SASPs contribute to spore resistance to peroxide and hypochlorite but may not be the only factors involved, since the coats and cortex also play a role (428).

Two other aspects of spores should be considered: the revival of injured spores and the effects of antiseptics and disinfectants on germinating and outgrowing spores. Although neither aspect is truly a resistance mechanism, each can provide useful information about the site and mechanism of action of sporicidal agents and about the associated spore resistance mechanisms and might be of clinical importance.

The revival of disinfectant-treated spores has not been extensively studied. Spicher and Peters (483, 484) demonstrated that formaldehyde-exposed spores of *B. subtilis* could be revived after a subsequent heat shock process. A more recent finding with *B. stearothermophilus* casts further doubt on the efficacy of low-temperature steam with formaldehyde as a sterilizing procedure (541). The revival of spores exposed to glutaraldehyde, formaldehyde, chlorine, and iodine was examined by Russell and his colleagues (103, 376, 377, 424, 532–537). A small proportion of glutaraldehyde-treated spores of various *Bacillus* species were revived when the spores were treated with alkali after neutralization of glutaraldehyde with glycine (103, 379, 380). Experiments designed to distinguish between germination and outgrowth in the revival process have demonstrated that sodium hydroxide-induced revival increases the potential for germination. Based on other findings, the germination process is also implicated in the revival of spores exposed to other disinfectants.

Intrinsic resistance of mycobacteria. Mycobacteria are well known to possess a resistance to antiseptics and disinfectants that is roughly intermediate between those of other nonsporulating bacteria and bacterial spores (Fig. 1) (177, 345, 419). There is no evidence that enzymatic degradation of harmful molecules takes place. The most likely mechanism for the high resistance of mycobacteria is associated with their complex cell walls that provide an effective barrier to the entry of these agents. To date, plasmid- or transposon-mediated resistance to biocides has not been demonstrated in mycobacteria.

The mycobacterial cell wall is a highly hydrophobic structure with a mycoylarabinogalactan-peptidoglycan skeleton (27, 105, 106, 322, 389, 390, 461, 530). The peptidoglycan is covalently linked to the polysaccharide copolymer (arabinogalactan) made up of arabinose and galactose esterified to mycolic acids. Also present are complex lipids, lipopolysaccharides (LPSs), and proteins, including porin channels through which hydrophilic molecules can diffuse into the cell (232, 356). Similar cell wall structures exist in all the mycobacterial species examined to date (228). The cell wall composition of a particular species may be influenced by its environmental niche (27). Pathogenic bacteria such as *Mycobacterium tuberculosis* exist in a relatively nutrient-rich environment, whereas saprophytic mycobacteria living in soil or water are exposed to natural antibiotics and tend to be more intrinsically resistant to these drugs.

Antiseptics or disinfectants that exhibit mycobacterial activity are phenol, PAA, hydrogen peroxide, alcohol, and glutaraldehyde (16, 17, 99, 419, 425, 455). By contrast, other well-known bactericidal agents, such as chlorhexidine and QACs, are mycobacteristatic even when used at high concentrations (51, 52, 419, 425, 455). However, the activity of these can be substantially increased by formulation effects. Thus, a number of QAC-based products claim to have mycobacterial activity. For example, a newer formulation (Sactimed-I-Sinald) containing a mixture of alkyl polyguanides and alkyl QACs is claimed to be mycobactericidal (211, 353). However, there is some doubt whether the antibacterial agents had been properly quenched or neutralized to prevent carryover of inhibitory concentrations into recovery media.

Many years ago, it was proposed (T. H. Shen, cited in reference 99) that the resistance of mycobacteria to QACs was related to the lipid content of the cell wall. In support of this contention, *Mycobacterium phlei*, which has a low total cell lipid content, was more sensitive than *M. tuberculosis*, which has a higher lipid content. It was also noted that the resistance of various species of mycobacteria was related to the content of waxy material in the wall. It is now known that because of the highly hydrophobic nature of the cell wall, hydrophilic biocides are generally unable to penetrate the mycobacterial cell wall in sufficiently high concentrations to produce a lethal effect. However, low concentrations of antiseptics and disinfectants such as chlorhexidine must presumably traverse this permeability barrier, because the MICs are of the same order as those concentrations inhibiting the growth of nonmycobacterial strains such as *S. aureus*, although *M. avium-intracellulare* may be particularly resistant (51, 52). The component(s) of the mycobacterial cell wall responsible for the high biocide resistance are currently unknown, although some information is available. Inhibitors of cell wall synthesis increase the susceptibility of *M. avium* to drugs (391); inhibition of myocide C, arabinogalactan, and mycolic acid biosynthesis enhances drug susceptibility. Treatment of this organism with *m*-fluoro-DL-phenylalanine (*m*-FL-phe), which inhibits mycocide C synthesis, produces significant alterations in the outer cell wall layers (106). Ethambutol, an inhibitor of arabinogalactan (391, 501) and phospholipid (461, 462) synthesis, also disorganizes these layers. In addition, ethambutol induces the formation of ghosts without the dissolution of peptidoglycan (391). Methyl-4-(2-octadecylcyclopropen-1-yl) butanoate (MOCB) is a structural analogue of a key precursor in mycolic acid synthesis. Thus, the effects of MOCB on mycolic acid synthesis and *m*-FL-phe and ethambutol on outer wall biosynthetic processes leading to changes in cell wall architecture appear to be responsible for increasing the intracellular concentration of chemotherapeutic drugs. These findings support the concept of the cell wall acting as a permeability barrier to these drugs (425). Fewer studies have been made of the mechanisms involved in the resistance of mycobacteria to antiseptics and disinfectants. However, the activity of chlorhexidine and of a QAC, cetylpyridinium chloride, against *M. avium* and *M. tuberculosis* can be potentiated in the presence of ethambutol (52). From these data, it may be inferred that arabinogalactan is one cell wall component that acts as a permeability barrier to chlorhexidine and QACs. It is not possible, at present, to comment on other components, since these have yet to be investigated. It would be useful to have information about the uptake into the cells of these antiseptic agents in the presence and absence of different cell wall synthesis inhibitors.

One species of mycobacteria currently causing concern is *M. chelonae*, since these organisms are sometimes isolated from endoscope washes and dialysis water. One such strain was not killed even after a 60-min exposure to alkaline glutaraldehyde; in contrast, a reference strain showed a 5-log-unit reduction after a contact time of 10 min (519). This glutaraldehyde-resistant *M. chelonae* strain demonstrated an increased tolerance to PAA but not to NaDCC or to a phenolic. Other workers have also observed an above-average resistance of *M. chelonae* to glutaraldehyde and formaldehyde (72) but not to PAA (187, 294). The reasons for this high glutaraldehyde resistance are unknown. However, *M. chelonae* is known to adhere strongly to smooth surfaces, which may render cells within a biofilm less susceptible to disinfectants. There is no evidence to date that uptake of glutaraldehyde by *M. chelonae* is reduced.

Intrinsic resistance of other gram-positive bacteria. The cell wall of staphylococci is composed essentially of peptidoglycan and teichoic acid. Neither of these appears to act as an effective barrier to the entry of antiseptics and disinfectants. Since high-molecular-weight substances can readily traverse the cell wall of staphylococci and vegetative *Bacillus* spp., this may explain the sensitivity of these organisms to many antibacterial agents including QACs and chlorhexidine (411, 417, 422, 428, 451).

However, the plasticity of the bacterial cell envelope is a well-known phenomenon (381). Growth rate and any growth-limiting nutrient will affect the physiological state of the cells. Under such circumstances, the thickness and degree of cross-linking of peptidoglycan are likely to be modified and hence the cellular sensitivity to antiseptics and disinfectants will be altered. For example, Gilbert and Brown (171) demonstrated that the sensitivity of *Bacillus megaterium* cells to chlorhexidine and 2-phenoxyethanol is altered when changes in growth rate and nutrient limitation are made with chemostat-grown cells. However, lysozyme-induced protoplasts of these cells remained sensitive to, and were lysed by, these membrane-active agents. Therefore, the cell wall in whole cells is responsible for their modified response.

In nature, *S. aureus* may exist as mucoid strains, with the cells surrounded by a slime layer. Nonmucoid strains are killed more rapidly than mucoid strains by chloroxylenol, cetrimide, and chlorhexidine, but there is little difference in killing by phenols or chlorinated phenols (263); removal of slime by washing rendered the cells sensitive. Therefore, the slime plays a protective role, either as a physical barrier to disinfectant penetration or as a loose layer interacting with or absorbing the biocide molecules.

There is no evidence to date that vancomycin-resistant enterococci or enterococci with high-level resistance to aminoglycoside antibiotics are more resistant to disinfectants than are antibiotic-sensitive enterococcal strains (9, 11, 48, 319). However, enterococci are generally less sensitive to biocides than are staphylococci, and differences in inhibitory and bactericidal concentrations have also been found among enterococcal species (257).

Intrinsic resistance of gram-negative bacteria. Gram-negative bacteria are generally more resistant to antiseptics and disinfectants than are nonsporulating, nonmycobacterial gram-positive bacteria (Fig. 2) (428, 440, 441). Examples of MICs against gram-positive and -negative organisms are provided in Table 6. Based on these data, there is a marked difference in the sensitivity of *S. aureus* and *E. coli* to QACs (benzalkonium, benzethonium, and cetrimide), hexachlorophene, diamidines, and triclosan but little difference in chlorhexidine susceptibility. *P. aeruginosa* is considerably more resistant to most of these agents, including chlorhexidine, and (not shown) *Proteus* spp. possess an above-average resistance to cationic agents such as chlorhexidine and QACs (311, 440).

The outer membrane of gram-negative bacteria acts as a barrier that limits the entry of many chemically unrelated types

TABLE 7. Possible transport of some antiseptics and disinfectants into gram-negative bacteria[a]

Antiseptic/disinfectant	Passage across OM[b]	Passage across IM[b]
Chlorhexidine	Self-promoted uptake(?)	IM is a major target site; damage to IM enables biocide to enter cytosol, where further interaction occurs
QACs	Self-promoted uptake(?); also, OM might present a barrier	IM is a major target site; damage to IM enables biocide to enter cytosol, where further interaction occurs
Phenolics	Hydrophobic pathway (activity increases as hydrophobicity of phenolic increases)	IM is a major target site, but high phenolic concentrations coagulate cytoplasmic constituents

[a] Data from references 197, 433 to 435, 438, and 439.
[b] OM, outer membrane; IM, inner membrane.

of antibacterial agents (18, 169, 196, 197, 355, 366, 440, 516, 517). This conclusion is based on the relative sensitivities of staphylococci and gram-negative bacteria and also on studies with outer membrane mutants of *E. coli*, *S. typhimurium*, and *P. aeruginosa* (134, 135, 433–435, 438). Smooth, wild-type bacteria have a hydrophobic cell surface; by contrast, because of the phospholipid patches on the cell surface, deep rough (heptose-less) mutants are hydrophobic. These mutants tend to be hypersensitive to hydrophobic antibiotics and disinfectants. Low-molecular-weight (M_r <ca. 600) hydrophilic molecules readily pass via the porins into gram-negative cells, but hydrophobic molecules diffuse across the outer membrane bilayer (Table 7). In wild-type gram-negative bacteria, intact LPS molecules prevent ready access of hydrophobic molecules to phospholipid and thence to the cell interior. In deep rough strains, which lack the O-specific side chain and most of the core polysaccharide, the phospholipid patches at the cell surface have their head groups oriented toward the exterior.

In addition to these hydrophilic and hydrophobic entry pathways, a third pathway has been proposed for cationic agents such as QACs, biguanidies, and diamidines. It is claimed that these damage the outer membrane, thereby promoting their own uptake (197). Polycations disorganize the outer membrane of *E. coli* (520). It must be added, however, that the QACs and diamidines are considerably less active against wild-type strains than against deep rough strains whereas chlorhexidine has the same order of activity (MIC increase about 2 to 3 fold) against both types of *E. coli* strains (434, 435, 439). However, *S. typhimurium* mutants are more sensitive to chlorhexidine than are wild-type strains (433).

Gram-negative bacteria that show a high level of resistance to many antiseptics and disinfectants include *P. aeruginosa*, *Burkholderia cepacia*, *Proteus* spp., and *Providencia stuartii* (428, 440). The outer membrane of *P. aeruginosa* is responsible for its high resistance; in comparison with other organisms, there are differences in LPS composition and in the cation content of the outer membrane (54). The high Mg^{2+} content aids in producing strong LPS-LPS links; furthermore, because of their small size, the porins may not permit general diffusion through them. *B. cepacia* is often considerably more resistant in the hospital environment than in artificial culture media (360); the high content of phosphate-linked arabinose in its LPS decreases the affinity of the outer membrane for polymyxin antibiotics and other cationic and polycationic molecules (97, 516). *Pseudomonas stutzeri*, by contrast, is highly sensitive to many antibiotics and disinfectants (449), which implies that such agents have little difficulty in crossing the outer layers of the cells of this organism.

Members of the genus *Proteus* are invariably insensitive to chlorhexidine (311). Some strains that are highly resistant to chlorhexidine, QACs, EDTA, and diamidines have been isolated from clinical sources. The presence of a less acidic type of outer membrane LPS could be a contributing factor to this intrinsic resistance (97, 516).

A particularly troublesome member of the genus *Providencia* is *P. stuartii*. Like *Proteus* spp., *P. stuartii* strains have been isolated from urinary tract infections in paraplegic patients and are resistant to different types of antiseptics and disinfectants including chlorhexidine and QACs (492, 496). Strains of *P. stuartii* that showed low-, intermediate-, and high-level resistance to chlorhexidine formed the basis of a series of studies of the resistance mechanism(s) (86, 422, 428). Gross differences in the composition of the outer layers of these strains were not detected, and it was concluded that (i) subtle changes in the structural arrangement of the cell envelopes of these strains was associated with this resistance and (ii) the inner membrane was not implicated (230).

Few authors have considered peptidoglycan in gram-negative bacteria as being a potential barrier to the entry of inhibitory substances. The peptidoglycan content of these organisms is much lower than in staphylococci, which are inherently more sensitive to many antiseptics and disinfectants. Nevertheless, there have been instances (discussed in reference 422) where gram-negative organisms grown in subinhibitory concentrations of a penicillin have deficient permeability barriers. Furthermore, it has been known for many years (215, 409, 411) that penicillin-induced spheroplasts and lysozyme-EDTA-Tris "protoplasts" of gram-negative bacteria are rapidly lysed by membrane-active agents such as chlorhexidine. It is conceivable that the stretched nature of both the outer and inner membranes in β-lactam-treated organisms could contribute to this increased susceptibility.

The possibility exists that the cytoplasmic (inner) membrane provides one mechanism of intrinsic resistance. This membrane is composed of lipoprotein and would be expected to prevent passive diffusion of hydrophilic molecules. It is also known that changes in membrane composition affect sensitivity to ethanol (159). Lannigan and Bryan (275) proposed that decreased susceptibility of *Serratia marcescens* to chlorhexidine was linked to the inner membrane, but Ismaeel et al. (230) could find no such role with chlorhexidine-resistant *P. stuartii*. At present, there is little evidence to implicate the inner membrane in biocide resistance. In addition, chlorhexidine degradation was reported for *S. marcescens*, *P. aeruginosa*, and *Achromobacter/Alcaligenes xylosoxidans* (358).

Physiological (phenotypic) adaption as an intrinsic mechanism. The association of microorganisms with solid surfaces leads to the generation of a biofilm, defined as a consortium of organisms organized within an extensive exopolysaccharide exopolymer (93, 94). Biofilms can consist of monocultures, of several diverse species, or of mixed phenotypes of a given species (57, 73, 381). Some excellent publications that deal with the nature, formation, and content of biofilms are available (125, 178, 276, 538). Biofilms are important for several reasons,

TABLE 8. Biofilms and microbial response to antimicrobial agents

Mechanism of resistance associated with biofilms	Comment
Exclusion or reduced access of antiseptic or disinfectant to underlying cell	Depends on (i) nature of antiseptic/disinfectant, (ii) binding capacity of glycocalyx toward antiseptic or disinfectant, and (iii) rate of growth of microcolony relative to diffusion rate of chemical inhibitor
Modulation of microenvironment	Associated with (i) nutrient limitation and (ii) growth rate
Increased production of degradative enzymes by attached cells	Mechanism unclear at present
Plasmid transfer between cells in biofilm?	Associated with enhanced tolerance to antiseptics and disinfectants?

notably biocorrosion, reduced water quality, and foci for contamination of hygienic products (10, 12–14). Colonization also occurs on implanted biomaterials and medical devices, resulting in increased infection rates and possible recurrence of infection (125).

Bacteria in different parts of a biofilm experience different nutrient environments, and their physiological properties are affected (57). Within the depths of a biofilm, for example, nutrient limitation is likely to reduce growth rates, which can affect susceptibility to antimicrobial agents (98, 142, 171, 172). Thus, the phenotypes of sessile organisms within biofilms differ considerably from the planktonic cells found in laboratory cultures (73). Slow-growing bacteria are particularly insusceptible, a point reiterated recently in another context (126).

Several reasons can account for the reduced sensitivity of bacteria within a biofilm (Table 8). There may be (i) reduced access of a disinfectant (or antibiotic) to the cells within the biofilm, (ii) chemical interaction between the disinfectant and the biofilm itself, (iii) modulation of the microenvironment, (iv) production of degradative enzymes (and neutralizing chemicals), or (v) genetic exchange between cells in a biofilm. However, bacteria removed from a biofilm and recultured in culture media are generally no more resistant than the "ordinary" planktonic cells of that species (57).

Several instances are known of the contamination of antiseptic or disinfectant solutions by bacteria. For example, Marrie and Costerton (310) described the prolonged survival of *S. marcescens* in 2% chlorhexidine solutions, which was attributed to the embedding of these organisms in a thick matrix that adhered to the walls of a storage containers. Similar conclusions were reached by Hugo et al. (225) concerning the survival of *B. cepacia* in chlorhexidine and by Anderson et al. (10, 12–14) concerning the contamination of iodophor antiseptics with *Pseudomonas*. In the studies by Anderson et al., *Pseudomonas* biofilms were found on the interior surfaces of polyvinyl chloride pipes used during the manufacture of providone-iodine antiseptics. It is to be wondered whether a similar reason could be put forward for the contamination by *S. marcescens* of a benzalkonium chloride solution implicated in meningitis (468). Recently, a novel strategy was described (540) for controlling biofilms through generation of hydrogen peroxide at the biofilm-surface interface rather than simply applying a disinfectant extrinsically. In this procedure, the colonized surface incorporated a catalyst that generated the active compound from a treatment agent.

Gram-negative pathogens can grow as biofilms in the catheterized bladder and are able to survive concentrations of chlorhexidine that are effective against organisms in noncatheterized individuals (493, 494). Interestingly, the permeability agent EDTA has only a temporary potentiating effect in the catheterized bladder, with bacterial growth subsequently recurring (495). *B. cepacia* freshly isolated from the hospital environment is often considerably more resistant to chlorhexidine than when grown in artificial culture media, and a glycocalyx may be associated with intrinsic resistance to the bisbiguanide (360). *Legionella pneumophila* is often found in hospital water distribution systems and cooling towers. Chlorination in combination with continuous heating (60°C) of incoming water is usually the most important disinfection measure; however, because of biofilm production, contaminating organisms may be less susceptible to this treatment (140). Increased resistance to chlorine has been reported for *Vibrio cholerae*, which expresses an amorphous exopolysaccharide causing cell aggregation ("rugose" morphology [336]) without any loss in pathogenicity.

One can reach certain conclusions about biofilms. The interaction of bacteria with surfaces is usually reversible and eventually irreversible. Irreversible adhesion is initiated by the binding of bacteria to the surface through exopolysaccharide glycocalyx polymers. Sister cells then arise by cell division and are bound within the glycocalyx matrix. The development of adherent microcolonies is thereby initiated, so that eventually a continuous biofilm is produced on the colonized surface. Bacteria within these biofilms reside in specific microenvironments that differ from those of cells grown under normal laboratory conditions and thus show variations in their response to antiseptics and disinfectants.

Recent nosocomial outbreaks due to *M. chelonae* (discussed under "Intrinsic resistance of mycobacteria"), *M. tuberculosis* (4, 323) and HCV (53) underscore the importance of pseudobiofilm formation in flexible fiberoptic scope contamination. These outbreaks were associated with inadequate cleaning of scopes, which compromised subsequent sterilization with glutaraldehyde. While these organisms do not form a true biofilm, the cross-linking action of glutaraldehyde can cause a buildup of insoluble residues and associated microorganisms on scopes and in automated reprocessors.

Biofilms provide the most important example of how physiological (phenotypic) adaptation can play a role in conferring intrinsic resistance (57). Other examples are also known. For example, fattened cells of *S. aureus* produced by repeated subculturing in glycerol-containing media are more resistant to alkyl phenols and benzylpenicillin than are wild-type strains (220). Subculture of these cells in routine culture media resulted in reversion to sensitivity (218). Planktonic cultures grown under conditions of nutrient limitation or reduced growth rates have cells with altered sensitivity to disinfectants, probably as a consequence of modifications in their outer membranes (56, 59, 98). In addition, many aerobic microorganisms have developed intrinsic defense systems that confer tolerance to peroxide stress (in particular H_2O_2) in vivo. The so-called oxidative-stress or SOS response has been well studied in *E. coli* and *Salmonella* and includes the production of neutralizing enzymes to prevent cellular damage (including peroxidases, catalases, glutathione reductase) and to repair DNA lesions (e.g., exonuclease III) (112, 114, 497). In both organisms, increased tolerance can be obtained by pretreatment with a subinhibitory dose of hydrogen peroxide (113, 539). Pretreatment induces a series of proteins, many of which are under the positive control of a sensor/regulator protein (OxyR), including catalase and glutathione reductase (497)

TABLE 9. Possible mechanisms of plasmid-encoded resistance to antiseptics and disinfectants

Chemical agent	Examples	Mechanisms
Antiseptics or disinfectants	Chlorhexidine salts	(i) Inactivation: not yet found to be plasmid mediated; chromosomally mediated inactivation; (ii) efflux: some *S. aureus*, some *S. epidermidis*; (iii) Decreased uptake(?)
	QACs	(i) Efflux: some *S. aureus*, some *S. epidermidis*; (ii) Decreased uptake(?)
	Silver compounds	Decreased uptake; no inactivation (cf. mercury compounds)
	Formaldehyde	(i) Inactivation by formaldehyde dehydrogenase; (ii) Cell surface alterations (outer membrane proteins)
	Acridines[a]	Efflux: some *S. aureus*, some *S. epidermidis*
	Diamidines	Efflux: some *S. aureus*, some *S. epidermidis*
	Crystal violet[a]	Efflux: some *S. aureus*, some *S. epidermidis*
Other biocides	Mercurials[b]	Inactivation (reductases, lyases)
	Ethidium bromide	Efflux: some *S. aureus*, some *S. epidermidis*

[a] Now rarely used for antiseptic or disinfectant purposes.
[b] Organomercurials are still used as preservatives.

and further nonessential proteins that accumulate to protect the cell (338). Cross-resistance to heat, ethanol, and hypochlorous acid has also been reported (81, 128, 335). The oxidative stress response in gram-positive bacteria is less well studied, but *Bacillus* tolerance to H_2O_2 has been described to vary during the growth phase (127) and in mutant strains (67, 200). Similar inducible defense mechanisms were described for *Campylobacter jejuni* (185), *Deinococcus* (528), and *Haemophilus influenzae* (36). However, the level of increased tolerance to H_2O_2 during the oxidative stress response may not afford significant protection to concentrations used in antiseptics and disinfectants (generally >3%). For example, *B. subtilis* mutants have been described to be more resistant at ~0.5% H_2O_2 than are wild-type strains at ~0.34% H_2O_2 (200).

Acquired Bacterial Resistance Mechanisms

As with antibiotics and other chemotherapeutic drugs, acquired resistance to antiseptics and disinfectants can arise by either mutation or the acquisition of genetic material in the form of plasmids or transposons. It is important to note that "resistance" as a term can often be used loosely and in many cases must be interpreted with some prudence. This is particularly true with MIC analysis. Unlike antibiotics, "resistance," or an increase in the MIC of a biocide, does not necessarily correlate with therapeutic failure. An increase in an antibiotic MIC can may have significant consequences, often indicating that the target organism is unaffected by its antimicrobial action. Increased biocide MICs due to acquired mechanisms have also been reported and in some case misinterpreted as indicating resistance. It is important that issues including the pleiotropic action of most biocides, bactericidal activity, concentrations used in products, direct product application, formulation effects, etc., be considered in evaluating the clinical implications of these reports.

Plasmids and bacterial resistance to antiseptics and disinfectants. Chopra (82, 83) examined the role of plasmids in encoding resistance (or increased tolerance) to antiseptics and disinfectants; this topic was considered further by Russell (413). It was concluded that apart from certain specific examples such as silver, other metals, and organomercurials, plasmids were not normally responsible for the elevated levels of antiseptic or disinfectant resistance associated with certain species or strains. Since then, however, there have been numerous reports linking the presence of plasmids in bacteria with increased tolerance to chlorhexidine, QACs, and triclosan, as well as to diamidines, acridines and ethidium bromide, and the topic was reconsidered (83, 423, 427) (Table 9).

Plasmid-encoded resistance to antiseptics and disinfectants had at one time been most extensively investigated with mercurials (both inorganic and organic), silver compounds, and other cations and anions. Mercurials are no longer used as disinfectants, but phenylmercuric salts and thiomersal are still used as preservatives in some types of pharmaceutical products (226). Resistance to mercury is plasmid borne, inducible, and may be transferred by conjugation or transduction. Inorganic mercury (Hg^{2+}) and organomercury resistance is a common property of clinical isolates of *S. aureus* containing penicillinase plasmids (110). Plasmids conferring resistance to mercurials are either narrow spectrum, specifying resistance to Hg^{2+} and to some organomercurials, or broad-spectrum, with resistance to the above compounds and to additional organomercurials (331). Silver salts are still used as topical antimicrobial agents (50, 443). Plasmid-encoded resistance to silver has been found in *Pseudomonas stutzeri* (192), members of the *Enterobacteriaceae* (479, 480, 511), and *Citrobacter* spp. (511). The mechanism of resistance has yet to be elucidated fully but may be associated with silver accumulation (152, 511).

(i) Plasmid-mediated antiseptic and disinfectant resistance in gram-negative bacteria. Occasional reports have examined the possible role of plasmids in the resistance of gram-negative bacteria to antiseptics and disinfectants. Sutton and Jacoby (498) observed that plasmid RP1 did not significantly alter the resistance of *P. aeruginosa* to QACs, chlorhexidine, iodine, or chlorinated phenols, although increased resistance to hexachlorophene was observed. This compound has a much greater effect on gram-positive than gram-negative bacteria, so that it is difficult to assess the significance of this finding. Transformation of this plasmid (which encodes resistance to carbenicillin, tetracycline, and neomycin and kanamycin) into *E. coli* or *P. aeruginosa* did not increase the sensitivity of these organisms to a range of antiseptics (5).

Strains of *Providencia stuartii* may be highly tolerant to Hg^{2+}, cationic disinfectants (such as chlorhexidine and QACs), and antibiotics (496). No evidence has been presented to show that there is a plasmid-linked association between antibiotic resistance and disinfectant resistance in these organisms, pseudomonads, or *Proteus* spp. (492). High levels of disinfectant resistance have been reported in other hospital isolates (195), although no clear-cut role for plasmid-specified resistance has emerged (102, 250, 348, 373, 518). High levels of tolerance to chlorhexidine and QACs (311) may be intrinsic or may have resulted from mutation. It has been proposed (492, 496) that the extensive usage of these cationic agents could be responsible for the selection of antiseptic-disinfectant-, and antibiotic-resistant strains; however, there is little evidence to support this conclusion. All of these studies demonstrated that it was difficult to transfer chlorhexidine or QAC resistance under nor-

TABLE 10. *qac* genes and susceptibility of *S. aureus* strains to some antiseptics and disinfectants

qac gene[a]	MIC ratios[b] of[c]:							
	Proflavine	CHG	Pt	Pi	CTAB	BZK	CPC	DC
qacA	>16	2.5	>16	>16	4	>3	>4	2
qacB	8	1	>4	2	2	>3	>2	2
qacC	1	1	ca. 1	1	6	>3	>4	1
qacD	1	1	ca. 1	1	6	>3	>4	1
MIC (μg/ml) for sensitive strain	40	0.8	<50	50[d]	1	<2	<1	4

[a] *qac* genes are otherwise known as nucleic acid binding (NAB) compound resistance genes (88).

[b] Calculated from the data in reference 289. Ratios are MICs for strains of *S. aureus* carrying various *qac* genes divided by the MIC for a strain carrying no gene (the actual MIC for the test strain is shown in the bottom row).

[c] CHG, chlorhexidine diacetate; Pt, pentamidine isethionate; Pi, propamidime isethionate; CTAB, cetyltrimethylammonium bromide; BZK, benzalkonium chloride; CPC, cetylpyridinium chloride; DC, dequalinium chloride.

[d] The MIC of propamidine isethionate for the sensitive *S. aureus* is considerably higher than the normal quoted value (ca. 2 μg/ml [Table 6]).

mal conditions and that plasmid-mediated resistance to these chemicals in gram-negative bacteria was an unlikely event. By contrast, plasmid R124 alters the OmpF outer membrane protein in *E. coli*, and cells containing this plasmid are more resistant to a QAC (cetrimide) and to other agents (406).

Bacterial resistance mechanisms to formaldehyde and industrial biocides may be plasmid encoded (71, 193). Alterations in the cell surface (outer membrane proteins [19, 246]) and formaldehyde dehydrogenase (247, 269) are considered to be responsible (202). In addition, the so-called TOM plasmid encodes enzymes for toluene and phenol degradation in *B. cepacia* (476).

(ii) Plasmid-mediated antiseptic and disinfectant resistance in staphylococci. Methicillin-resistant *S. aureus* (MRSA) strains are a major cause of sepsis in hospitals throughout the world, although not all strains have increased virulence. Many can be referred to as "epidemic" MRSA because of the ease with which they can spread (91, 295, 317). Patients at particularly high risk are those who are debilitated or immunocompromised or who have open sores.

It has been known for several years that some antiseptics and disinfectants are, on the basis of MICs, somewhat less inhibitory to *S. aureus* strains that contain a plasmid carrying genes encoding resistance to the aminoglycoside antibiotic gentamicin (Table 10). These biocidal agents include chlorhexidine, diamidines, and QACs, together with ethidium bromide and acridines (8, 238, 289, 368, 423, 427, 463). According to Mycock (346), MRSA strains showed a remarkable increase in tolerance (at least 5,000-fold) to povidone-iodine. However, there was no decrease in susceptibility of antibiotic-resistant strains to phenolics (phenol, cresol, and chlorocresol) or to the preservatives known as parabens (8).

Tennent et al. (505) proposed that increased resistances to cetyltrimethylammonium bromide (CTAB) and to propamidine isethionate were linked and that these cationic agents may be acting as a selective pressure for the retention of plasmids encoding resistance to them. The potential clinical significance of this finding remains to be determined.

Staphylococci are the only bacteria in which the genetic aspects of plasmid-mediated antiseptic and disinfectant resistant mechanisms have been described (466). In *S. aureus*, these mechanisms are encoded by at least three separate multidrug resistance determinants (Tables 10 and 11). Increased antiseptic MICs have been reported to be widespread among MRSA strains and to be specified by two gene families (*qacAB* and *qacCD*) of determinants (188, 280, 281, 288, 289, 363–365, 367, 506). The *qacAB* family of genes (Table 11) encodes proton-dependant export proteins that develop significant homology to other energy-dependent transporters such as the tetracycline transporters found in various strains of tetracycline-resistant bacteria (405). The *qacA* gene is present predominantly on the pSK1 family of multiresistance plasmids but is also likely to be present on the chromosome of clinical *S. aureus* strains as an integrated family plasmid or part thereof. The *qacB* gene is detected on large heavy-metal resistance plasmids. The *qacC* and *qacD* genes encode identical phenotypes and show restriction site homology; the *qacC* gene may have evolved from *qacD* (288).

Interesting studies by Reverdy et al. (395, 396), Dussau et al. (129) and Behr et al. (31) demonstrated a relationship between increased *S. aureus* MICs to the β-lactam oxacillin and four antiseptics (chlorhexidine, benzalkonium chloride, hexamine, and acriflavine). A gene encoding multidrug resistance was not found in susceptible strains but was present in 70% of *S. aureus* strains for which the MICs of all four of these antiseptics were increased and in 45% of the remaining strains resistant to at least one of these antiseptics (31). Genes encoding increased QAC tolerance may be widespread in food-associated staphylococcal species (203). Some 40% of isolates of coagulase-negative staphylococci (CNS) contain both *qacA* and *qacC* genes, with a possible selective advantage in possessing both as opposed to *qacA* only (281). Furthermore, there is growing evidence that *S. aureus* and CNS have a common pool of resistance determinants.

Triclosan is used in surgical scrubs, soaps, and deodorants. It is active against staphylococci and less active against most gram-negative organisms, especially *P. aeruginosa*, probably by virtue of a permeability barrier (428). Low-level transferable resistance to triclosan was reported in MRSA strains (88, 90); however, no further work on these organisms has been described. According to Sasatsu et al. (465), the MICs of triclosan against sensitive and resistant *S. aureus* strains were 100 and

TABLE 11. *qac* genes and resistance to quaternary ammonium compounds and other antiseptics and disinfectants

Multidrug resistance determinant[a]	Gene location	Resistance encoded to
qacA	pSK1 family of multiresistant plasmids, also β-lactamase and heavy-metal resistance families	QACs, chlorhexidine salts, diamidines, acridines, ethidium bromide
qacB	β-Lactamase and heavy-metal resistance plasmids	QACs, acridines, ethidium bromide
qacC[b]	Small plasmids (<3 kb) or large conjugative plasmids	Some QACs, ethidium bromide
qacD[b]	Large (50-kb) conjugative, multiresistance plasmids	Some QACs, ethidium bromide

[a] The *qacK* gene has also been described, but it is likely to be less significant than *qacAB* in terms of antiseptic or disinfectant tolerance.

[b] These genes have identical target sites and show restriction site homology.

>6,400 μg/ml, respectively; these results were disputed because these concentrations are well in excess of the solubility of triclosan (515), which is practically insoluble in water. Sasatsu et al. (464) described a high-level resistant strain of *S. aureus* for which the MICs of chlorhexidine, CTAB, and butylparaben were the same as for a low-level resistant strain. Furthermore, the MIC quoted for methylparaben comfortably exceeds its aqueous solubility. Most of these studies with sensitive and "resistant" strains involved the use of MIC evaluations (for example, Table 6). A few investigations examined the bactericidal effects of antiseptics. Cookson et al. (89) pointed out that curing of resistance plasmids produced a fall in MICs but not a consistent decrease in the lethal activity of chlorhexidine. There is a poor correlation between MIC and the rate of bactericidal action of chlorhexidine (88, 89, 319) and triclosan (90, 319). McDonnell et al. (318, 319) have described methicillin-susceptible *S. aureus* (MSSA) and MRSA strains with increased triclosan MICs (up to 1.6 μg/ml) but showed that the MBCs for these strains were identical; these results were not surprising, considering that biocides (unlike antibiotics) have multiple cellular targets. Irizarry et al. (229) compared the susceptibility of MRSA and MSSA strains to CPC and chlorhexidine by both MIC and bactericidal testing methods. However, the conclusion of this study that MRSA strains were more resistant warrants additional comments. On the basis of rather high actual MICs, MRSA strains were some four times more resistant to chlorhexidine and five times more resistant to a QAC (CPC) than were MSSA strains. At a concentration in broth of 2 μg of CPC/ml, two MRSA strains grew normally with a threefold increase in viable numbers over a 4-h test period whereas an MSSA strain showed a 97% decrease in viability. From this, the authors concluded that it was reasonable to speculate that the residual amounts of antiseptics and disinfectants found in the hospital environment could contribute to the selection and maintenance of multiresistant MRSA strains. Irizarry et al. (229) also concluded that MRSA strains are less susceptible than MSSA strains to both chronic and acute exposures to antiseptics and disinfectants. However, their results with 4 μg of CPC/ml show no such pattern: at this higher concentration, the turbidities (and viability) of the two MRSA and one MSSA strains decreased at very similar rates (if anything, one MRSA strain appeared to be affected to a slightly greater extent that the MSSA strain). Furthermore, the authors stated that chlorhexidine gave similar results to CPC. It is therefore difficult to see how Irizarry et al. arrived at their highly selective conclusions.

Plasmid-mediated efflux pumps are particularly important mechanisms of resistance to many antibiotics (85), metals (349), and cationic disinfectants and antiseptics such as QACs, chlorhexidine, diamidines, and acridines, as well as to ethidium bromide (239, 289, 324–336, 363–368). Recombinant *S. aureus* plasmids transferred into *E. coli* are responsible for conferring increased MICs of cationic agents to the gram-negative organism (505, 544). Midgley (324, 325) demonstrated that a plasmid-borne, ethidium resistance determinant from *S. aureus* cloned in *E. coli* encodes resistance to ethidium bromide and to QACs, which are expelled from the cells. A similar efflux system is present in *Enterococcus hirae* (326).

Sasatsu et al. (463) showed that duplication of *ebr* is responsible for resistance to ethidium bromide and to some antiseptics. Later, Sasatsu et al. (466) examined the origin of *ebr* (now known to be identical to *qacCD*) in *S. aureus*; *ebr* was found in antibiotic-resistant and -sensitive strains of *S. aureus*, CNS, and enterococcal strains. The nucleotide sequences of the amplified DNA fragment from sensitive and resistant strains were identical, and it was proposed that in antiseptic-resistant cells there was an increase in the copy number of the *ebr* (*qacCD*) gene whose normal function was to remove toxic substances from normal cells of staphylococci and enterococci.

Based on DNA homology, it was proposed that *qacA* and related genes carrying resistance determinants evolved from preexisting genes responsible for normal cellular transport systems (405) and that the antiseptic resistance genes evolved before the introduction and use of topical antimicrobial products and other antiseptics and disinfectants (288, 289, 365, 367, 368, 405).

Baquero et al. (23) have pointed out that for antibiotics, the presence of a specific resistance mechanism frequently contributes to the long-term selection of resistant variants under in vivo conditions. Whether low-level resistance to cationic antiseptics, e.g., chlorhexidine, QACs, can likewise provide a selective advantage on staphylococci carrying *qac* genes remains to be elucidated. The evidence is currently contentious and inconclusive.

(iii) Plasmid-mediated antiseptic and disinfectant resistance in other gram-positive bacteria. Antibiotic-resistant corynebacteria may be implicated in human infections, especially in the immunocompromised. 'Group JK' coryneforms (*Corynebacterium jeikeium*) were found to be more tolerant than other coryneforms to the cationic disinfectants ethidium bromide and hexachlorophene, but studies with plasmid-containing and plasmid-cured derivatives produced no evidence of plasmid-associated resistance (285). *Enterococcus faecium* strains showing high level resistance to vancomycin, gentamicin, or both are not more resistant to chlorhexidine or other nonantibiotic agents (9, 11, 20, 319). Furthermore, despite the extensive dental use of chlorhexidine, strains of *Streptococcus mutans* remain sensitive to it (235). To date, therefore, there is little or no evidence of plasmid-associated resistance of nonstaphylococcal gram-positive bacteria to antiseptics and disinfectants.

Mutational resistance to antiseptics and disinfectants. Chromosomal mutation to antibiotics has been recognized for decades. By contrast, fewer studies have been performed to determine whether mutation confers resistance to antiseptics and disinfectants. It was, however, demonstrated over 40 years ago (77, 78) that *S. marcescens*, normally inhibited by QACs at <100 μg/ml, could adapt to grow in 100,000 μg of a QAC per ml. The resistant and sensitive cells had different surface characteristics (electrophoretic mobilities), but resistance could be lost when the cells were grown on QAC-free media. One problem associated with QACs and chlorhexidine is the turbidity produced in liquid culture media above a certain concentration (interaction with agar also occurs), which could undoubtedly interfere with the determination of growth. This observation is reinforced by the findings presented by Nicoletti et al. (354).

Prince et al. (383) reported that resistance to chlorhexidine could be induced in some organisms but not in others. For example, *P. mirabilis* and *S. marcescens* displayed 128- and 258-fold increases, respectively, in resistance to chlorhexidine, whereas it was not possible to develop resistance to chlorhexidine in *Salmonella enteritidis*. The resistant strains did not show altered biochemical properties of changed virulence for mice, and some strains were resistant to the QAC benzalkonium chloride. Moreover, resistance to chlorhexidine was stable in *S. marcescens* but not in *P. mirabilis*. Despite extensive experimentation with a variety of procedures, Fitzgerald et al. (148) were unable to develop stable chlorhexidine resistance in *E. coli* or *S. aureus*. Similar observations were made by Cookson et al. (89), who worked with MRSA and other strains of *S. aureus*, and by McDonnell et al. (319), who worked with MRSA and enterococci. Recently, stable chlorhexidine resistance was developed in *P. stutzeri* (502); these strains showed

various levels of increased tolerance to QACs, triclosan, and some antibiotics, probably as a result of a nonspecific alteration of the cell envelope (452). The adaptation and growth of *S. marcescens* in contact lens disinfectants containing chlorhexidine, with cross-resistance to a QAC, have been described previously (166).

Chloroxylenol-resistant strains of *P. aeruginosa* were isolated by repeated exposure in media containing gradually increasing concentrations of the phenolic, but the resistance was unstable (432). The adaptation of *P. aeruginosa* to QACs is a well-known phenomenon (1, 2, 240). Resistance to amphoteric surfactants has also been observed, and, interestingly, cross-resistance to chlorhexidine has been noted (240). This implies that the mechanism of such resistance is nonspecific and that it involves cellular changes that modify the response of organisms to unrelated biocidal agents. Outer membrane modification is an obvious factor and has indeed been found with QAC-resistant and amphoteric compound-resistant *P. aeruginosa* (240) and with chlorhexidine-resistant *S. marcescens* (166). Such changes involve fatty acid profiles and, perhaps more importantly, outer membrane proteins. It is also pertinent to note here the recent findings of Langsrud and Sundheim (274). In this study, resistance of *P. fluorescens* to QACs was reduced when EDTA was present with the QAC (although the lethal effect was mitigated after the cells were grown in medium containing QAC and EDTA); similar results have been found with laboratory-generated *E. coli* mutants for which the MICs of triclosan were increased (318). EDTA has long been known (175, 410) to produce changes in the outer membrane of gram-negative bacteria, especially pseudomonads. Thus, it appears that, again, the development of resistance is associated with changes in the cell envelope, thereby limiting uptake of antiseptics and disinfectants.

Hospital (as for other environmental) isolates of gram-negative bacteria are invariably less sensitive to disinfectants than are laboratory strains (196, 209, 279, 286, 492). Since plasmid-mediated transfer has apparently been ruled out (see above), selection and mutation could play an important role in the presence of these isolates. Subinhibitory antibiotic concentrations may cause subtle changes in the bacterial outer structure, thereby stimulating cell-to-cell contact (109); it remains to be tested if residual concentrations of antiseptics or disinfectants in clinical situations could produce the same effect.

Another insusceptibility mechanism has been put forward, in this instance to explain acridine resistance. It has been proposed (270, 351) that proflavine-sensitive and -resistant cells are equally permeable to the acridine but that resistant cells possessed the ability to expel the bound dye. This is an important point and one that has been reinforced by more recent studies that demonstrate the significance of efflux in resistance of bacteria to antibiotics (284, 330, 355). Furthermore, multidrug resistance (MDR) is a serious problem in enteric and other gram-negative bacteria. MDR is a term used to describe resistance mechanisms used by genes that form part of the normal cell genome (168). These genes are activated by induction or mutation caused by some types of stress, and because they are distributed ubiquitously, genetic transfer is not needed. Although such systems are most important in the context of antibiotic resistance, George (168) provides several examples of MDR systems in which an operon or gene is associated with changes in antiseptic or disinfectant susceptibility; e.g., (i) mutations at an *acr* locus in the Acr system render *E. coli* more sensitive to hydrophobic antibiotics, dyes, and detergents; (ii) the *robA* gene is responsible for overexpression in *E. coli* of the RobA protein that confers multiple antibiotic and heavy-metal resistance (interestingly, Ag^+ may be effluxed [350]); and (iii) the MarA protein controls a set of genes (*mar* and *soxRS* regulons) that confer resistance not only to several antibiotics but also to superoxide-generating agents. Moken et al. (333) have found that low concentrations of pine oil (used as a disinfectant) could select for *E. coli* mutants that overexpressed MarA and demonstrated low levels of cross-resistance to antibiotics. Deletion of the *mar* or *acrAB* locus (the latter encodes a PMF-dependant efflux pump) increased the susceptibility of *E. coli* to pine oil; deletion of *acrAB*, but not of *mar*, increased the susceptibility of *E. coli* to chloroxylenol and to a QAC. In addition, the *E. coli* MdfA (multidrug transporter) protein was recently identified and confers greater tolerance to both antibiotics and a QAC (benzalkonium) (132). The significance of these and other MDR systems in bacterial susceptibility to antiseptics and disinfectants, in particular the issue of cross-resistance with antibiotics, must be studied further. At present, it is difficult to translate these laboratory findings to actual clinical use, and some studies have demonstrated that antibiotic-resistant bacteria are not significantly more resistant to the lethal (or bactericidal) effects of antiseptic and disinfectants than are antibiotic-sensitive strains (11, 88, 89, 319).

TABLE 12. Possible mechanisms of fungal resistance to antiseptics and disinfectants

Type of resistance	Possible mechanism	Example(s)
Intrinsic	Exclusion	Chlorhexidine
	Enzymatic inactivation	Formaldehyde
	Phenotypic modulation	Ethanol
	Efflux	Not demonstrated to date[a]
Acquired	Mutation	Some preservative
	Inducible efflux	Some preservatives[a]
	Plasmid-mediated responses	Not demonstrated to date

[a] Efflux is now known to be one mechanism of fungal resistance to antibiotics (531).

Mechanisms of Fungal Resistance to Antiseptics and Disinfectants

In comparison with bacteria, very little is known about the ways in which fungi can circumvent the action of antiseptics and disinfectants (104, 111, 296). There are two general mechanisms of resistance (Table 12): (i) intrinsic resistance, a natural property or development of an organism (201); and (ii) acquired resistance. In one form of intrinsic resistance, the cell wall presents a barrier to reduce or exclude the entry of an antimicrobial agent. The evidence to date is somewhat patchy, but the available information links cell wall glucan, wall thickness, and relatively porosity to the susceptibility of *Saccharomyces cerevisiae* to chlorhexidine (Table 13) (204–208). Protoplasts of this organism prepared by glucuronidase in the presence of β-mercaptoethanol are lysed by chlorhexidine concentrations well below those effective against "normal" (whole) cells. Furthermore, culture age influences the response of *S. cerevisiae* to chlorhexidine; the cells walls are much less sensitive at stationary phase than at logarithmic growth phase (208), taking up much less [^{14}C]chlorhexidine gluconate (206). Gale (165) demonstrated a phenotypic increase in the resistance of *Candida albicans* to the polyenic antibiotic amphotericin B as the organisms entered the stationary growth phase, which was attributed to cell wall changes involving tighter cross-linking (74). Additionally, any factor increasing glucanase activity increased amphotericin sensitivity.

The porosity of the yeast cell wall is affected by its chemical

TABLE 13. Parameters affecting the response of *S. cerevisiae* to chlorhexidine[a]

Parameter	Role in susceptibility of cells to chlorhexidine
Cell wall composition	
Mannan	No role found to date
Glucan	Possible significance: at concentrations below those active against whole cells, chlorhexidine lyses protoplasts
Cell wall thickness	Increases in cells of older cultures: reduced chlorhexidine uptake responsible for decreased activity(?)
Relative porosity	Decreases in cells of older cultures: reduced chlorhexidine uptake responsible for decreased activity(?)
Plasma membrane	Changes altering CHG susceptibility(?); not investigated to date

[a] Data from references 204 to 208 and 436.

composition, with the wall acting as a barrier or modulator to the entry and exit of various agents. DeNobel et al. (117–119) used the uptake of fluorescein isothiocyanurate (FITC) dextrans and the periplasmic enzyme invertase as indicators of yeast cell wall porosity. Intact *S. cerevisiae* cells were able to endocytose FITC dextrans of 70 but not of 150. A new assay for determining the relative cell wall porosity in yeast based upon polycation-induced leakage of UV-absorbing compounds was subsequently developed. Hiom et al. (206, 208) found that the relative porosity of cells decreases with increasing culture age and that there was a reduced uptake of radiolabeled chlorhexidine gluconate. As the age of an *S. cerevisiae* culture increases, there is a significant increase in the cell wall thickness, with values of 0.19, 0.25, and 0.31 μm recorded for cells from 1-, 2-, and 6-day old cultures, respectively (206).

These findings (Table 13) can provide a tentative picture of the cellular factors that modify the response of *S. cerevisiae* to chlorhexidine. Mannan mutants of *S. cerevisiae* show a similar degree of sensitivity to chlorhexidine as the parent strain (204). The glucan layer is shielded from β-glucuronidase by mannoproteins, but this effect is overcome by β-mercaptoethanol (119). The mannoprotein consists of two fractions, sodium dodecyl sulfate-soluble mannoproteins and sodium dodecyl sulfate-insoluble, glucanase-soluble ones: the latter limit cell wall porosity (119). Thus, glucan (and possibly mannoproteins) plays a key role in determining the uptake and hence the activity of chlorhexidine in *S. cerevisiae*. *C. albicans* is less sensitive and takes up less [^{14}C]chlorhexidine overall (206), but only a few studies with this organism and with molds have been performed.

Yeasts grown under different conditions have variable levels of sensitivity to ethanol (176, 402). Cells with linoleic acid-enriched plasma membranes are more resistant to ethanol than are cells with oleic acid-enriched ones, from which it has been inferred that a more fluid membrane enhances ethanol resistance (6).

There is no evidence to date of antiseptic efflux (although benzoic acid in energized cells is believed to be eliminated by flowing down the electrochemical gradient [529]) and no evidence of acquired resistance by mutation (except to some preservatives [436]) or by plasmid-mediated mechanisms (426, 436). It is disappointing that so few rigorous studies have been performed with yeasts and molds and antiseptics and disinfectants (see also Miller's [328] treatise on mechanisms for reaching the site of action). Molds are generally more resistant than yeasts (Table 14) and considerably more resistant than nonsporulating bacteria (Table 15). Mold spores, although more resistant than nonsporulating bacteria, are less resistant than bacterial spores to antiseptics and disinfectants (436). It is tempting to speculate that the cell wall composition in molds confers a high level of intrinsic resistance on these organisms.

Mechanisms of Viral Resistance to Antiseptics and Disinfectants

Early studies on the effects of disinfectants on viruses were reviewed by Grossgebauer (189). Potential viral targets are the viral envelope, which contains lipids and is a typical unit membrane; the capsid, which is principally protein in nature; and the genome. An important hypothesis was put forward in 1963 (258) and modified in 1983 (259) in which it was proposed that viral susceptibility to disinfectants could be based on whether viruses were "lipophilic" in nature, because they possessed a lipid envelope (e.g., herpes simplex virus [259]) or "hydrophilic" because they did not (e.g., poliovirus [514]). Lipid-enveloped viruses were sensitive to lipophilic-type disinfectants, such as 2-phenylphenol, cationic surfactants (QACs), chlorhexidine, and isopropanol, as well as to ether and chloroform. Klein and Deforest (259) further classified viruses into three groups (Table 16), A (lipid containing), B (nonlipid picornaviruses), and C (other nonlipid viruses larger than those in group B) and disinfectants into two groups, broad-spectrum ones that inactivated all viruses and lipophilic ones that failed to inactivate picornoviruses and parvoviruses.

Capsid proteins are predominantly protein in nature, and biocides such as glutaraldehyde, hypochlorite, ethylene oxide, and hydrogen peroxide, which react strongly with amino or sulfhydryl groups might possess virucidal activity. It must, however, be added that destruction of the viral capsid may result in the release of a potentially infectious nucleic acid and that viral inactivation would only be complete if the viral nucleic acid is also destroyed.

Unfortunately, the penetration of antiseptics and disinfectants into different types of viruses and their interaction with viral components have been little studied, although some information has been provided by investigations with bacteriophages (307). Bacteriophages are being considered as "indicator species" for assessing the virucidal activity of disinfectants (108) and could thus play an increasing important role in this context; for example, repeated exposure of *E. coli* phage f2 to chlorine was claimed to increase its resistance to disinfection (542).

Thurman and Gerber (509, 510) pointed out that conflicting results on the actions of disinfectants on different virus types were often reported, and they suggested that the structural integrity of a virus was altered by an agent that reacted with viral capsids to increase viral permeability. Thus, a "two-stage"

TABLE 14. Lethal concentrations of antiseptics and disinfectants toward some yeasts and molds[a]

Antimicrobial agent[b]	Lethal concn (μg/μl) toward:		
	Yeast (*Candida albicans*)	Molds	
		Penicillium chrysogenum	*Aspergillus niger*
QACs			
Benzalkonium chloride	10	100–200	100–200
Cetrimide/CTAB	25	100	250
Chlorhexidine	20–40	400	200

[a] Derived in part from data in reference 525.
[b] CTAB, cetyltrimethylammonium bromide.

TABLE 15. Kinetic approach: *D*-values at 20°C of phenol and benzalkonium chloride against fungi and bacteria[a]

Antimicrobial agent	pH	Concn (%, wt/vol)	*D*-value (h)[b] against:				
			Aspergillus niger	*Candida albicans*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Phenol	5.1	0.5	20	13.5	0.94	—[c]	0.66
	6.1	0.5	32.4	18.9	1.72	0.17	1.9
Benzalkonium chloride	5.1	0.001	—[d]	9.66	0.06	3.01	3.12
	6.1	0.002	—[d]	5.5	—[c]	0.05	0.67

[a] Abstracted from the data in references 244 and 245.
[b] *D*-values are the times to reduce the viable population by 1 log unit.
[c] Inactivation was so rapid that the *D*-values could not be measured.
[d] No inactivation: fungistatic effect only.

disinfection system could be an efficient means of viral inactivation while overcoming the possibility of multiplicity reactivation (first put forward by Luria [293]) to explain an initial reduction and then an increase in the titer of disinfectant-treated bacteriophage. Multiplicity reactivation as a mechanism of resistance was supported by the observation of Young and Sharp (546) that clumping of poliovirus following partial inactivation by hypochlorite significantly increased the phage titer. It is envisaged as consisting of random damage to the capsid protein or nucleic acid of clumped, noninfectious virions from which complementary reconstruction of an infectious particle occurs by hybridization with the gene pool of the inactivated virions (298).

Another resistance mechanism also involves viral aggregation, e.g., the persistence of infectivity of formaldehyde-treated poliovirus (458) and the resistance of Norwalk virus to chlorination (249). A typical biphasic survival curve of enterovirus and rotavirus exposed to peracetic acid is also indicative of the presence of viral aggregates (198).

Finally, there remains the possibility of viral adaptation to new environmental conditions. In this context, Bates et al. (28) described the development of poliovirus having increased resistance to chlorine inactivation. Clearly, much remains to be learned about the mechanism of viral inactivation by and viral resistance to disinfectants.

Mechanisms of Protozoal Resistance to Antiseptics and Disinfectants

Intestinal protozoa, such as *Cryptosporidium parvum*, *Entamoeba histolytica*, and *Giardia intestinalis*, are all potentially pathogenic to humans and have a resistant, transmissible cyst (or oocyst for *Cryptosporidium*) (233, 234). Of the disinfectants available currently, ozone is the most effective protozoan cysticide, followed by chlorine dioxide, iodine, and free chlorine, all of which are more effective than the chloramines (234, 264). Cyst forms are invariably the most resistant to chemical disinfectants (Fig. 1). The reasons for this are unknown, but it would be reasonable to assume that cysts, similar to spores, take up fewer disinfectant molecules from solution than do vegetative forms.

Some recent studies have compared the responses of cysts and trophozoites of *Acanthamoeba castellanii* to disinfectants used in contact lens solutions and monitored the development of resistance during encystation and the loss of resistance during excystation (251–255). The lethal effects of chlorhexidine and of a polymeric biguanide were time and concentration dependent, and mature cysts were more resistant than preencystment trophozoites or preexcystment cysts. The cyst "wall" appeared to act as a barrier to the uptake of these agents, thereby presenting a classical type of intrinsic resistance mechanism (163). Acanthamoebae are capable of forming biofilms on surfaces such as contact lenses (186). Although protozoal biofilms have yet to be studied extensively in terms of their response to disinfectants, it is apparent that they could play a significant role in modulating the effects of chemical agents.

Mechanisms of Prion Resistance to Disinfectants

The transmissible degenerative encephalopathies (TDEs) form a group of fatal neurological diseases of humans and other animals. TDEs are caused by prions, abnormal proteinaceous agents that appear to contain no agent-specific nucleic acid (385). An abnormal protease-resistant form (PrP^{res}) of a normal host protein is implicated in the pathological process.

Prions are considered highly resistant to physical and chemical agents (Fig. 1), although the fact that crude preparations are often studied means that extraneous materials could, at least to some extent, mask the true efficacy of these agents (503). According to Taylor (503), there is currently no known decontamination procedure that will guarantee the complete absence of infectivity in TDE-infected tissues processed by histopathological procedures. Prions survive acid treatment, but a synergistic effect with autoclaving plus sodium hydroxide treatment is observed. Formaldehyde, unbuffered glutaraldehyde (acidic pH), and ethylene oxide have little effect on infectivity, although chlorine-releasing agents (especially hypochlorites), sodium hydroxide, some phenols, and guanidine thiocyanate are more effective (141, 309, 503).

With the information presently available, it is difficult to explain the extremely high resistance of prions, save to comment that the protease-resistant protein is abnormally stable to degradative processes.

CONCLUSIONS

It is clear that microorganisms can adapt to a variety of environmental physical and chemical conditions, and it is therefore not surprising that resistance to extensively used antiseptics and disinfectants has been reported. Of the mechanisms that have been studied, the most significant are clearly intrinsic, in particular the ability to sporulate, adaptation of pseudomonads, and the protective effects of biofilms. In these cases, "resistance" may be incorrectly used and "tolerance," defined as developmental or protective effects that permit microorganisms to survive in the presence of an active agent, may be more correct. Many of these reports of resistance have often paralleled issues including inadequate cleaning, incorrect product use, or ineffective infection control practices, which cannot be underestimated. Some acquired mechanisms (in particular with heavy-metal resistance) have also been shown to be clinically significant, but in most cases the results have been spec-

TABLE 16. Viral classification and response to some disinfectants[a]

Viral group	Lipid envelope[b]	Examples of viruses	Effects of disinfectants[c]	
			Lipophilic	Broad-spectrum
A	+	HSV, HIV, Newcastle disease virus, rabies virus, influenza virus	S	S
B	−	Non-lipid picornaviruses (poliovirus, Coxsackie virus, echovirus)	R	S
C	−	Other larger nonlipid viruses (adenovirus, reovirus)	R	S

[a] Data from reference 259; see also reference 444. For information on the inactivation of poliovirus, see reference 514.
[b] Present (+) or absent (−).
[c] Lipophilic disinfectants include QACs and chlorhexidine. S, sensitive; R, resistant.

ulative. Increased MICs have been confirmed, in particular for staphylococci. However, few reports have further investigated increased bactericidal concentrations or actual use dilutions of products, which in many cases contain significantly higher concentrations of biocides, or formulation attributes, which can increase product efficacy; in a number of cases, changes in the MICs have actually been shown not to be significant (9, 88, 89, 319, 428). Efflux mechanisms are known to be important in antibiotic resistance, but it is questionable if the observed increased MICs of biocides could have clinical implications for hard-surface or topical disinfection (423, 428). It has been speculated that low-level resistance may aid in the survival of microorganisms at residual levels of antiseptics and disinfectants; any possible clinical significance of this remains to be tested. With growing concerns about the development of biocide resistance and cross-resistance with antibiotics, it is clear that clinical isolates should be under continual surveillance and possible mechanisms should be investigated.

It is also clear that antiseptic and disinfectant products can vary significantly, despite containing similar levels of biocides, which underlines the need for close inspection of efficacy claims and adequate test methodology (183, 423, 428). In addition, a particular antiseptic or disinfectant product may be better selected (as part of infection control practices) based on particular circumstances or nosocomial outbreaks; for example, certain active agents are clearly more efficacious against gram-positive than gram-negative bacteria, and *C. difficile* (despite the intrinsic resistance of spores) may be effectively controlled physically by adequate cleaning with QAC-based products.

In conclusion, a great deal remains to be learned about the mode of action of antiseptics and disinfectants. Although significant progress has been made with bacterial investigations, a greater understanding of these mechanisms is clearly lacking for other infectious agents. Studies of the mechanisms of action of and microbial resistance to antiseptics and disinfectants are thus not merely of academic significance. They are associated with the more efficient use of these agents clinically and with the potential design of newer, more effective compounds and products.

REFERENCES

1. **Adair, F. W., S. G. Geftic, and J. Gelzer.** 1969. Resistance of *Pseudomonas* to quaternary ammonium compounds. I. Growth in benzalkonium chloride solution. Appl. Microbiol. **18**:299–302.
2. **Adair, F. W., S. G. Geftic, and J. Gelzer.** 1971. Resistance of *Pseudomonas* to quaternary ammonium compounds. II. Cross resistance characteristics of a mutant of *Pseudomonas aeruginosa*. Appl. Microbiol. **21**:1058–1063.
3. **Adler-Storthz, K., L. M. Schulster, G. R. Dreesman, F. B. Hollinger, and J. L. Melnick.** 1983. Effect of alkaline glutaraldehyde on hepatitis B virus antigens. Eur. J. Clin. Microbiol. **2**:316–320.
4. **Agerton, T., S. Valway, B. Gore, C. Pozsik, B. Plikaytis, C. Woodley, and I. Onorato.** 1997. Transmission of a highly drug-resistant strain (strain W1) of *Mycobacterium tuberculosis*. JAMA **278**:1073–1077.
5. **Ahonkhai, I., and A. D. Russell.** 1979. Response RP1$^+$ and RP1$^-$ strains of *Escherichia coli* to antibacterial agents and transfer of resistance to *Pseudomonas aeruginosa*. Curr. Microbiol. **3**:89–94.
6. **Alexandre, H., I. Rousseaux, and C. Charpentier.** 1994. Relationship between ethanol tolerance, lipid composition and plasma membrane fluidity in *Saccharomyces cerevisiae* and *Kloeckera apiculata*. FEMS Microbiol. Lett. **124**:17–22.
7. **Alfa, M. J., and D. L. Sitter.** 1994. In-hospital evaluation of orthophthaldehyde as a high level disinfectant for flexible endoscopes. J. Hosp. Infect. **26**:15–26.
8. **Al-Masaudi, S. B., M. J. Day, and A. D. Russell.** 1991. Antimicrobial resistance and gene transfer in *Staphylococcus aureus*. J. Appl. Bacteriol. **70**: 279–290.
9. **Alqurashi, A. M., M. J. Day, and A. D. Russell.** 1996. Susceptibility of some strains of enterococci and streptococci to antibiotics and biocides. J. Antimicrob. Chemother. **38**:745.
10. **Anderson, R. L.** 1989. Iodophor antiseptics: intrinsic microbial contamination with resistant bacteria. Infect. Control Hosp. Epidemiol. **10**:443–446.
11. **Anderson, R. L., J. H. Carr, W. W. Bond, and M. S. Favero.** 1997. Susceptibility of vancomycin-resistant enterococci to environmental disinfectants. Infect. Control Hosp. Epidemiol. **18**:195–199.
12. **Anderson, R. L., B. W. Holland, J. K. Carr, W. W. Bond, and M. S. Favero.** 1990. Effect of disinfectants on pseudomonads colonized on the interior surface of PVC pipes. Am. J. Public Health **80**:17–21.
13. **Anderson, R. L., R. W. Vess, J. H. Carr, W. W. Bond, A. L. Panlilio, and M. S. Favero.** 1991. Investigations of intrinsic *Pseudomonas cepacia* contamination in commercially manufactured povidone-iodine. Infect. Control Hosp. Epidemiol. **12**:297–302.
14. **Anderson, R. L., R. W. Vess, A. L. Panlilio, and M. S. Favero.** 1990. Prolonged survival of *Pseudomonas cepacia* in commercially manufactured povidone-iodine. Appl. Environ. Microbiol. **56**:3598–3600.
15. **Apostolov, K.** 1980. The effects of iodine on the biological activities of myxoviruses. J. Hyg. **84**:381–388.
16. **Ascenzi, J. M.** 1996. Glutaraldehyde-based disinfectants, p. 111–132. *In* J. M. Ascenzi (ed.), Handbook of disinfectants and antiseptics. Marcel Dekker, Inc., New York, N.Y.
17. **Ayliffe, G. A. J., D. Coates, and P. N. Hoffman.** 1993. Chemical disinfection in hospitals, 2nd ed. Public Health Laboratory, London, England.
18. **Ayres, H., J. R. Furr, and A. D. Russell.** 1993. A rapid method of evaluating permeabilizing activity against *Pseudomonas aeruginosa*. Lett. Appl. Microbiol. **17**:149–151.
19. **Azachi, M., Y. Henis, R. Shapira, and A. Oren.** 1996. The role of the outer membrane in formaldehyde tolerance in *Escherichia coli* VU3695 and *Halomonas* sp. MAC. Microbiology **142**:1249–1254.
20. **Baillie, L. W. J., J. J. Wade, and M. W. Casewell.** 1992. Chlorhexidine sensitivity of *Enterococcus faecium* resistant to vancomycin, high levels of gentamicin, or both. J. Hosp. Infect. **20**:127–128.
21. **Bailly, J.-L., M. Chambron, H. Peigue-Lafeuille, H. Laveran, C. de Champs, and D. Beytout.** 1991. Activity of glutaraldehyde at low concentrations (<2%) against poliovirus and its relevance to gastrointestinal endoscope disinfection procedures. Appl. Environ. Microbiol. **57**:1156–1160.
22. **Baldry, M. G. C., and J. A. L. Fraser.** 1988. Disinfection with peroxygens. Crit. Rep. Appl. Chem. **22**:91–116.
23. **Baquero, F., C. Patron, R. Canton, and M. M. Ferrer.** 1991. Laboratory and in-vitro testing of skin antiseptics: a prediction for in-vitro activity. J. Hosp. Infect. **18**(Suppl. B):5–11.
24. **Barett-Bee, K., L. Newboult, and S. Edwards.** 1994. The membrane destabilizing action of the antibacterial agent chlorhexidine. FEMS Microbiol. Lett. **119**:249–254.
25. **Barkvoll, P., and G. Rolla.** 1994. Triclosan protects the skin against dermatitis caused by sodium lauryl sulphate exposure. Clin. Periodontol. **21**: 717–719.
26. **Barrette, W. C., Jr., D. M. Hannum, W. D. Wheeler, and J. K. Hurst.** 1989. General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. Biochemistry **28**:9172–9178.
27. **Barry, C. E., III, and K. Mdluli.** 1996. Drug sensitivity and environmental adaptation of mycobacterial cell wall components. Trends Microbiol. **4**: 275–281.
28. **Bates, R. C., P. T. B. Schaffer, and S. M. Sutherland.** 1977. Development of poliovirus having increased resistance to chlorine inactivation. Appl. Environ. Microbiol. **3**:849–853.
29. **Bayliss, C. E., W. M. Waites, and N. R. King.** 1981. Resistance and structure of spores of *Bacillus subtilis*. J. Appl. Bacteriol. **50**:379–390.
30. **Beaver, D. J., D. P. Roman, and P. J. Stoffel.** 1957. The preparation and

bacteriostatic activity of substituted ureas. J. Am. Chem. Soc. 79:1236–1245.
31. **Behr, H., M. E. Reverdy, C. Mabilat, J. Freney, and J. Fleurette.** 1994. Relation entre le niveau des concentrations minimales inhibitrices de cinq antiseptiques et la présence du gène *qac*A chez *Staphylococcus aureus*. Pathol. Biol. 42:438–444.
32. **Belly, R. T., and G. C. Kydd.** 1982. Silver resistance in microorganisms. Dev. Ind. Microbiol. 23:567–577.
33. **Benarde, M. A., W. B. Snow, V. P. Olivieri, and B. Davidson.** 1967. Kinetics and mechanism of bacterial disinfection by chlorine dioxide. Appl. Microbiol. 15:257–265.
34. **Best, M., V. S. Springthorpe, and S. A. Sattar.** 1994. Feasibility of a combined carrier test for disinfectants: studies with a mixture of five types of micro-organisms. Am. J. Infect. Control 22:152–162.
35. **Beveridge, E. G., I. Boyd, I. Dew, M. Haswell, and C. W. G. Lowe.** 1991. Electron and light microscopy of damaged bacteria. Soc. Appl. Bacteriol. Tech. Ser. 27:135–153.
36. **Bishai, W. R., H. O. Smith, and G. J. Barcak.** 1994. A peroxide/ascorbate-inducible catalase from *Haemophilus influenzae* is homologous to the *Escherichia coli katE* gene product. J. Bacteriol. 176:2914–2921.
37. **Black, J. G., D. Howes, and T. Rutherford.** 1975. Skin deposition and penetration of trichlorocarbanilide. Toxicology 3:253–264.
38. **Block, S. S.** 1991. Peroxygen compounds, p. 167–181. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
39. **Block, S. S.** 1991. Historical review, p. 3–17. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
40. **Block, S. S.** 1991. Definitions of terms, p. 18–125. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
41. **Bloomfield, S. F.** 1974. The effect of the phenolic antibacterial agent Fentichlor on energy coupling in *Staphylococcus aureus*. J. Appl. Bacteriol. 37: 117–131.
42. **Bloomfield, S. F.** 1996. Chlorine and iodine formulations, p. 133–158. *In* J. M. Ascenzi (ed.), Handbook of disinfectants and antiseptics. Marcel Dekker, Inc., New York, N.Y.
43. **Bloomfield, S. F.** Resistance of bacterial spores to chemical agents. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
44. **Bloomfield, S. F., and M. Arthur.** 1992. Interaction of *Bacillus subtilis* spores with sodium hypochlorite, sodium dichloroisocyanurate and chloramine-T. J. Appl. Bacteriol. 72:166–172.
45. **Bloomfield, S. F., and M. Arthur.** 1994. Mechanisms of inactivation and resistance of spores to chemical biocides. J. Appl. Bacteriol. Symp. Suppl. 76:91S–104S.
46. **Bloomfield, S. F., C. A. Smith-Burchnell, and A. G. Dalgleish.** 1990. Evaluation of hypochlorite-releasing disinfectants against the human immunodeficiency virus (HIV). J. Hosp. Infect. 15:273–278.
47. **Bobichon, H., and P. Bouchet.** 1987. Action of chlorhexidine on budding *Candida albicans*: scanning and transmission electron microscopic study. Mycopathologia 100:27–35.
48. **Bradley, C. R., and A. P. Fraise.** 1996. Heat and chemical resistance in enterococci. J. Hosp. Infect. 34:191–196.
49. **Bragg, P. D., and D. J. Rannie.** 1974. The effect of silver ions on the respiratory chain of *Escherichia coli*. Can. J. Microbiol. 20:883–889.
50. **Bridges, K., and E. J. L. Lowbury.** 1977. Drug resistance in relation to use of silver sulphadiazine cream in a burn unit. J. Clin. Pathol. 31:160–164.
51. **Broadley, S. J., P. A. Jenkins, J. R. Furr, and A. D. Russell.** 1991. Antimycobacterial activity of biocides. Lett. Appl. Microbiol. 13:118–122.
52. **Broadley, S. J., P. A. Jenkins, J. R. Furr, and A. D. Russell.** 1995. Potentiation of the effects of chlorhexidine diacetate and cetylpyridinium chloride on mycobacteria by ethambutol. J. Med. Microbiol. 43:458–460.
53. **Bronowicki, J. P., V. Venard, C. Botte, N. Monhoven, I. Gastin, L. Chone, H. Hudziak, B. Rhin, C. Delanoe, A. LeFaou, M.-A. Bigard, and P. Gaucher.** 1997. Patient-to-patient transmission of hepatitis C virus during colonoscopy. N. Engl. J. Med. 337:237–240.
54. **Brown, M. R. W.** 1975. The role of the cell envelope in resistance, p. 71–99. *In* M. R. W. Brown (ed.), Resistance of *Pseudomonas aeruginosa*. John Wiley & Sons, Ltd., Chichester, England.
55. **Brown, M. R. W., and R. A. Anderson.** 1968. The bactericidal effect of silver ions on *Pseudomonas aeruginosa*. J. Pharm. Pharmacol. 20(Suppl.):1S–3S.
56. **Brown, M. R. W., P. J. Collies, and P. Gilbert.** 1990. Influence of growth rate on susceptibility to antimicrobial agents: modification of the cell envelope and batch and continuous culture studies. Antimicrob. Agents Chemother. 34:1623–1628.
57. **Brown, M. R. W., and P. Gilbert.** 1993. Sensitivity of biofilms to antimicrobial agents. J. Appl. Bacteriol. Symp. Suppl. 74:87S–97S.
58. **Brown, M. R. W., and J. Melling.** 1969. Loss of sensitivity to EDTA by *Pseudomonas aeruginosa* grown under conditions of Mg limitation. J. Gen. Microbiol. 54:439–444.
59. **Brown, M. R. W., and P. Williams.** 1985. The influence of environment on envelope properties affecting survival of bacteria in infections. Annu. Rev. Microbial. 39:527–556.
60. **Brown, T. A., and D. G. Smith.** 1976. The effects of silver nitrate on the growth and ultrastructure of the yeast *Cryptococcus albidus*. Microbios Lett. 3:155–162.
61. **Broxton, P., P. M. Woodcock, and P. Gilbert.** 1983. A study of the antibacterial activity of some polyhexamethylene biguanides towards *Escherichia coli* ATCC 8739. J. Appl. Bacteriol. 54:345–353.
62. **Broxton, P., P. M. Woodcock, and P. Gilbert.** 1984. Interaction of some polyhexamethylene biguanides and membrane phospholipids in *Escherichia coli*. J. Appl. Bacteriol. 57:115–124.
63. **Broxton, P., P. M. Woodcock, and P. Gilbert.** 1984. Injury and recovery of *Escherichia coli* ATCC 8739 from treatment with some polyhexamethylene biguanides. Microbios 40:187–193.
64. **Broxton, P., P. M. Woodcock, and P. Gilbert.** 1984. Binding of some polyhexamethylene biguanides to the cell envelope of *Escherichia coli* ATCC 8739. Microbios 41:15–22.
65. **Bruck, C. W.** 1991. Role of glutaraldehyde and other liquid chemical sterilants in the processing of new medical devices, p. 376–396. *In* R. F. Morrissey and Y. I. Prokopenko (ed.), Sterilization of medical products, vol. V. Polyscience Publications, Morin Heights, Canada.
66. **Bruch, M. K.** 1996. Chloroxylenol: an old-new antimicrobial, p. 265–294. *In* J. M. Ascenzi (ed.), Handbook of disinfectants and antiseptics. Marcel Dekker, Inc., New York, N.Y.
67. **Bsat, N., L. Chen, and J. D. Helmann.** 1996. Mutation of the *Bacillus subtilis* alkyl hydroperoxide reductase (*ahpCF*) operon reveals compensatory interactions among hydrogen peroxide stress genes. J. Bacteriol. 178:6579–6586.
68. **Bush, L. E., L. M. Benson, and J. H. White.** 1986. Pig skin as a test substrate for evaluating topical antimicrobial activity. J. Clin. Microbiol. 24:343–348.
69. **Cabral, J. P. S.** 1991. Mode of antibacterial action of dodine (dodecylguanidine monoacetate) in *Pseudomonas syringae*. Can. J. Microbiol. 38:115–123.
70. **Camper, A. K., and G. A. McFeters.** 1979. Chlorine injury and the enumeration of waterborne coliform bacteria. Appl. Environ. Microbiol. 37:633–641.
71. **Candal, F. J., and R. G. Eagon.** 1984. Evidence for plasmid-mediated bacterial resistance to industrial biocides. Int. Biodeterior. Biodegrad. 20: 221–224.
72. **Carson, L. A., N. J. Petersen, M. S. Favero, and S. M. Aguero.** 1978. Growth characteristics of atypical mycobacteria in water and their comparative resistance to disinfectants. Appl. Environ. Microbiol. 36:839–846.
73. **Caspentier, B., and O. Cerf.** 1993. Biofilms and their consequences, with particular reference to hygiene in the food industry. J. Appl. Bacteriol. 75: 499–511.
74. **Cassone, A., D. Kerridge, and E. F. Gale.** 1979. Ultrastructural changes in the cell wall of *Candida albicans* following the cessation of growth and their possible relationship to the development of polyene resistance. J. Gen. Microbiol. 110:339–349.
75. **Chambon, M., J.-L. Bailly, and H. Peigue-Lafeuille.** 1992. Activity of gluteraldehyde at low concentrations against capsid proteins of poliovirus type 1 and echovirus type 25. Appl. Environ. Microbiol. 58:3517–3521.
76. **Chang, S. L.** 1971. Modern concept of disinfection. J. Sanit. Eng. Div. Proc. ASCE 97:689.
77. **Chaplin, C. E.** 1951. Observations on quaternary ammonium disinfectants. J. Bot. 29:373–382.
78. **Chaplin, C. E.** 1952. Bacterial resistance to quaternary ammonium disinfectants. J. Bacteriol. 63:453–458.
79. **Chawner, J. A., and P. Gilbert.** 1989. A comparative study of the bactericidal and growth inhibitory activities of the bisbiguanides alexidine and chlorhexidine. J. Appl. Bacteriol. 66:243–252.
80. **Chawner, J. A., and P. Gilbert.** 1989. Interaction of the bisbiguanides chlorhexidine and alexidine with phospholipid vesicles: evidence for separate modes of action. J. Appl. Bacteriol. 66:253–258.
81. **Chesney, J., J. W. Eaton, and J. R. Mahoney, Jr.** 1996. Bacterial glutathione: a sacrificial defense against chlorine compounds. J. Bacteriol. 178: 2131–2135.
82. **Chopra, I.** 1982. Plasmids and bacterial resistance, p. 199–206. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization. Blackwell Scientific Publications Ltd., Oxford, England.
83. **Chopra, I.** 1987. Microbial resistance to veterinary disinfectants and antiseptics, p. 43–65. *In* A. H. Linton, W. B. Hugo, and A. D. Russell (ed.), Disinfection in veterinary and farm animal practice. Blackwell Scientific Publications Ltd., Oxford, England.
84. **Chopra, I.** 1991. Bacterial resistance to disinfectants, antiseptics and toxic metal ions. Soc. Appl. Bacteriol. Tech. Ser. 27:45–64.
85. **Chopra, I.** 1992. Efflux-based antibiotic resistance mechanisms: the evidence for increasing prevalence. J. Antimicrob. Chemother. 30:737–739.
86. **Chopra, I., S. C. Johnson, and P. M. Bennett.** 1987. Inhibition of *Providencia stuartii* cell envelope enzymes by chlorhexidine. J. Antimicrob. Chemother. 19:743–751.
87. **Christensen, E. A., and H. Kristensen.** 1991. Gaseous sterilization, p. 557–

572. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (eds.), Principles and practice of disinfection, preservation and sterilization, 2nd ed. Blackwell Scientific Publications Ltd., Oxford, England.
88. **Cookson, B. D.** 1994. Antiseptic resistance in methicillin-resistant *Staphylococcus aureus*: an emerging problem? p. 227–234. *In* Proceedings of the 7th International Symposium on Staphylococci and Staphlococcal Infections. Gustav Fischer Verlag, Stuttgart, Germany.
89. **Cookson, B. D., M. C. Bolton, and J. H. Platt.** 1991. Chlorhexidine resistance in *Staphylococcus aureus* or just an elevated MIC? An in vitro and in vivo assessment. Antimicrob. Agents Chemother. 35:1997–2002.
90. **Cookson, B. D., H. Farrelly, M.-F. Palepou, and R. George.** 1992. Transferable resistance to triclosan in MRSA. Lancet 337:1548–1549.
91. **Cookson, B. D., and I. Phillips.** 1988. Epidemic methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. 21(Suppl. C):57–65.
92. **Corner, T. R., H. L. Joswick, J. N. Silvernale, and P. Gerhardt.** 1971. Antimicrobial actions of hexachlorophane: lysis and fixation of bacterial protoplasts. J. Bacteriol. 108:501–507.
93. **Costerton, J. D., Z. Lewandowski, D. DeBeer, D. Caldwell, D. Korber, and G. James.** 1994. Biofilms, the customized niche. J. Bacteriol. 176:2137–2142.
94. **Costerton, J. W., K.-J. Cheng, G. G. Geesey, T. I. Ladd, J. C. Nickel, M. Dasgupta, and T. J. Marrie.** 1987. Bacterial biofilms in nature and disease. Annu. Rev. Microbiol. 41:435–464.
95. **Coulthard, C. E., and G. Skyes.** 1936. Germicidal effect of alcohol. Pharm. J. 137:79–81.
96. **Coward, J. S., H. S. Carr, and H. S. Rosenkranz.** 1973. Silver sulfadiazine effect on the ultrastructure of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 3:621–624.
97. **Cox, A. D., and S. G. Wilkinson.** 1991. Ionizing groups of lipopolysaccharides of *Pseudomonas cepacia* in relation to antibiotic resistance. Mol. Microbiol. 5:641–646.
98. **Cozens, R. M., and M. R. W. Brown.** 1983. Effect of nutrient depletion on the sensitivity of *Pseudomonas cepacia* to antimicrobial agents. J. Pharm. Sci. 72:1363–1365.
99. **Croshaw, B.** 1971. The destruction of mycobacteria, p. 420–449. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Ltd., London, England.
100. **Crow, S.** 1992. Peracetic acid sterilization: a timely development for a busy healthcare industry. Infect. Control Hosp. Epidemiol. 13:111–113.
101. **Dagely, S., E. A. Dawes, and G. A. Morrison.** 1950. Inhibition of growth of *Aerobacter aerogenes*: the mode of action of phenols, alcohols, acetone and ethyl acetate. J. Bacteriol. 60:369–378.
102. **Dance, D. A. B., A. D. Pearson, D. V. Seal, and J. A. Lowes.** 1987. A hospital outbreak caused by a chlorhexidine and antibiotic resistant *Proteus mirabilis*. J. Hosp. Infect. 10:10–16.
103. **Dancer, B. N., E. G. M. Power, and A. D. Russell.** 1989. Alkali-reduced revival of *Bacillus* spores after inactivation by glutaraldehyde. FEMS Microbiol. Lett. 57:345–348.
104. **D'Arcy, P. F.** 1971. Inhibition and destruction of moulds and yeats, p. 613–686. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Ltd., London, England.
105. **David, H. L., N. Rastogi, S. Clavel-Sérès, F. Clément, and M.-F. Thorel.** 1987. Structure of the cell envelope of *Mycobacterium avium*. Zentbl. Bakteriol. Mikrobiol. Hyg. Ser. A 264:49–66.
106. **David, H. L., N. Rastogi, S. Clavel-Sérès, F. Clément.** 1988. Alterations in the outer wall architecture caused by the inhibition of mycoside C biosynthesis in *Mycobacterium avium*. Curr. Microbiol. 17:61–68.
107. **Davies, A., and B. S. Field.** 1969. Action of biguanides, phenol and detergents on *Escherichia coli* and its spheroplasts. J. Appl. Bacteriol. 32:233–243.
108. **Davies, J.** 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science 264:375–382.
109. **Davies, J. G., J. R. Babb, C. R. Bradley, and G. A. J. Ayliffe.** 1993. Preliminary study of test methods to assess the virucidal activity of skin disinfectants using poliovirus and bacteriophages. J. Hosp. Infect. 25:125–131.
110. **Day, M. J., and A. D. Russell.** Antibiotic-resistant cocci. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
111. **Dekker, J.** 1987. Development of resistance to modern fungicides and strategies for its avoidance, p. 39–52. *In* H. Lyr (ed.), Modern slective fungicides. Longman, Harlow, England.
112. **Demple, B.** 1991. Regulation of bacterial oxidative stress genes. Annu. Rev. Genet. 25:315–337.
113. **Demple, B., and J. Halbrook.** 1983. Inducible repair of oxidative damage in *E. coli*. Nature 304:466–468.
114. **Demple, B., and L. Harrison.** 1994. Repair of oxidative damage to DNA: enzymology and biology. Annu. Rev. Biochem. 63:915–948.
115. **Dennis, W. H., V. P. Olivieri, and C. W. Kruse.** 1979. The reaction of nucleotides with aqueous hypochlorous acid. Water Res. 13:357–362.
116. **Dennis, W. H., V. P. Olivieri, and C. W. Kruse.** 1979. Mechanism of disinfection: incorporation of Cl-36 into f2 virus. Water Res. 13:363–369.
117. **De Nobel, J. G., C. Dijkers, E. Hooijberg, and F. M. Klis.** 1989. Increased cell wall porosity in *Saccharomyces cerevisiae* after treatment with dithiothreitol or EDTA. J. Gen. Microbiol. 135:2077–2084.
118. **De Nobel, J. G., F. M. Klis, T. Munnik, and H. Van Den Ende.** 1990. An assay of relative cell porosity in *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Schizosaccharomyces pombe*. Yeast 6:483–490.
119. **De Nobel, J. G., F. M. Klis, J. Priem, T. Munnik, and H. Van Den Ende.** 1990. The glucanase-soluble mannoproteins limit cell porosity in *Saccharomyces cerevisiae*. Yeast 6:491–499.
120. **Denyer, S. P.** 1995. Mechanisms of action of antibacterial biocides. Int. Biodeterior. Biodegrad. 36:227–245.
121. **Denyer, S. P., and W. B. Hugo.** 1977. The mode of action of cethyltrimethylammonium bromide (CTAB) on *Staphylococcus aureus*. J. Pharm. Pharmacol. 29:66P.
122. **Denyer, S. P., and W. B. Hugo.** 1991. Biocide-induced damage to the cytoplasmic membrane. Soc. Appl. Bacteriol. Tech. Ser. 27:171–187.
123. **Denyer, S. P., W. B. Hugo, and V. D. Harding.** 1985. Synergy in preservative combinations. Int. J. Pharm. 25:245–253.
124. **Denyer, S. P., W. B. Hugo, and V. D. Harding.** 1986. The biochemical basis of synergy between the antibacterial agents chlorocresol and 2-phenylethanol. Int. J. Pharm. 29:29–36.
125. **Denyer, S. P., S. P. Gorman, and M. Sussman.** 1993. Microbial biofilms: formation and control. Soc. Appl. Bacteriol. Tech. Ser. 30.
126. **Dodd, C. E. R., R. L. Sharman, S. F. Bloomfield, I. R. Booth, and G. S. A. B. Stewart.** 1997. Inimical processes: bacterial self-destruction and sub-lethal injury. Trends. Food Sci. Technol. 8:238–241.
127. **Dowds, B. C., P. Murphy, D. J. McConnell, and K. M. Devine.** 1987. Relationship among oxidative stress, growth cycle, and sporulation in *Bacillus subtilis*. J. Bacteriol. 169:5771–5775.
128. **Dukan, S., and D. Touati.** 1996. Hypochlorous acid stress in *Escherichia coli*: resistance, DNA damage, and comparison with hydrogen peroxide stress. J. Bacteriol. 178:6145–6150.
129. **Dussau, J. Y., J. C. Chapalain, Y. Rouby, M. E. Reverdy, and M. Bartoli.** 1993. Evaluation par une microméthode de l'activité bactericide de cinq disinfectancts sur 108 souches hospitalières. Pathol. Biol. 41:349–357.
130. **Dychdala, G. R.** 1991. Chlorine and chlorine compounds, p. 131–151. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
131. **Dye, M., and G. C. Mead.** 1972. The effect of chlorine on the viability of clostridial spores. J. Food Technol. 7:173–181.
132. **Edgar, R., and E. Bibi.** 1997. MdfA, and *Escherichia coli* multidrug resistance protein with an extraordinarily broad spectrum of drug recognition. J. Bacteriol. 179:2274–2280.
133. **Eklund, T., and I. F. Nes.** 1991. Effects of biocides on DNA, RNA and protein synthesis. Soc. Appl. Bacteriol. Tech. Ser. 27:225–234.
134. **El-Falaha, B. M. A., A. D. Russell, and J. R. Furr.** 1983. Sensitivities of wild-type and envelope-defective strains of *Escherichia coli* and *Pseudomonas aeruginosa* to antibacterial agents. Microbios 38:99–105.
135. **El-Falaha, B. M. A., A. D. Russell, and J. R. Furr.** 1985. Effect of chlorhexidine diacetate and benzalkonium chloride on the viability of wild-type and envelope mutants of *Escherichia coli* and *Pseudomonas aeruginosa*. Lett. Appl. Microbiol. 1:21–24.
136. **Elferink, J. G. R.** 1974. The effect of ethylenediamine tetraacetic acid on yeast cell membranes. Protoplasma 80:261–268.
137. **Elferink, J. G. R., and H. L. Booij.** 1974. Interaction of chlorhexidine with yeast cells. Biochem. Pharmacol. 23:1413–1419.
138. **Ellar, D. J., Munoz, and M. R. T. Salton.** 1971. The effect of low concentrations of glutaraldehyde on *Micrococcus lysodeikticus* membranes. Biochim. Biophys. Acta 225:140–150.
139. **El-Moug, T., D. T. Rogers, J. R. Furr, B. M. A. El-Falaha, and A. D. Russell.** 1985. Antiseptic-induced changes in the cell surface of a chlorhexidine-sensitive and a chlorhexidine-resistant strain of *Providencia stuartii*. J. Antimicrob. Chemother. 16:685–689.
140. **Elsmore, R. D. Legionella.** *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
141. **Ernst, D. R., and R. E. Race.** 1993. Comparative analysis of scrapie agent inactivation methods. J. Virol. Methods 41:193–201.
142. **Evans, D. J., D. G. Allison, M. R. W. Brown, and P. Gilbert.** 1990. Growth rate and the resistance of Gram-negative biofilms to cetrimide. J. Antimicrob. Chemother. 26:473–478.
143. **Favero, M. S., and W. W. Bond.** 1991. Chemical disinfection of medical surgical material, p. 617–641. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
144. **Feron, V. J., H. P. Til, F. de Vrijes, R. A. Wouterson, F. R. Cassee, and P. J. van Bladeren.** 1991. Aldehydes: occurrence, carcinogenicity potential, mechanism of action and risk assessment. Mutat. Res. 259:363–385.
145. **Fitzgerald, K. A., A. Davies, and A. D. Russell.** 1989. Uptake of ^{14}C-chlorhexidine diacetate to *Escherichia coli* and *Pseudomonas aeruginosa* and its release by azolectin. FEMS Microbiol. Lett. 60:327–332.
146. **Fitzgerald, K. A., A. Davies, and A. D. Russell.** 1992. Effect of chlorhexidine and phenoxyethanol on cell surface hydrophobicity of Gram-positive and

Gram-negative bacteria. Lett. Appl. Microbiol. 14:91–95.
147. **Fitzgerald, K. A., A. Davies, and A. D. Russell.** 1992. Mechanism of ation of chlorhexidine diacetate and phenoxyethanol singly and in combination against Gram-negative bacteria. Microbios 70:215–230.
148. **Fitzgerald, K. A., A. Davies, and A. D. Russell.** 1992. Sensitivity and resistance of *Escherichia coli* and *Staphylococcus aureus* to chlorhexidine. Lett. Appl. Microbiol. 14:33–36.
149. **Floyd, R. D., G. Sharp, and J. D. Johnson.** 1979. Inactivation by chlorine of single poliovirus particles in water. Environ. Sci. Technol. 13:438–442.
150. **Foegeding, P. M., and F. F. Busta.** 1983. Proposed mechanism for sensitization by hypochlorite treatment of *Clostridium botulinum* spores. Appl. Environ. Microbiol. 45:1374–1379.
151. **Foster, S. J.** 1994. The role and regulation of cell wall structural dynamics during differentiation of endoscope-forming bacteria. J. Appl. Bacteriol. Symp. Suppl. 76:25S–39S.
152. **Foster, T. J.** 1983. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev. 47:361–409.
153. **Fox, C. L., Jr.** 1983. Topical therapy and the development of silver sulfadiazine. Surg. Gynecol. Obst. 157:82–88.
154. **Fox, C. L., Jr., and S. M. Modak.** 1974. Mechanism of silver sulfadiazine action on burn wound infections. Antimicrob. Agents Chemother. 5:582–588.
155. **Fraenkel-Conrat, H.** 1961. Chemical modification of viral ribonucleic acid (RNA). Alkylating agents. Biochim. Biophys. Acta 49:169–180.
156. **Fraenkel-Conrat, H., M. Cooper, and H. S. Olcott.** 1945. The reaction of formaldehyde with proteins. J. Am. Chem. Soc. 67:950–954.
157. **Fraenkel-Conrat, H., and H. S. Olcott.** 1946. Reaction of formaldehyde with proteins. II. Participation of the guanidyl groups and evidence of cross-linking. J. Am. Chem. Soc. 68:34–37.
158. **Frederick, J. F., T. R. Corner, and P. Gerhardt.** 1974. Antimicrobial actions of hexachlorophane: inhibition of respiration in *Bacillus megaterium*. Antimicrob. Agents Chemother. 6:712–721.
159. **Fried, V. A., and A. Novick.** 1973. Organic solvents as probes for the structure and function of the bacterial membrane: effects of ethanol on the wild type and on an ethanol-resistant mutant of *Escherichia coli* K-12. J. Bacteriol. 114:239–248.
160. **Frier, M.** 1971. Derivatives of 4-amino-quinaldinium and 8-hydroxyquinoline, p. 107–120. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Ltd., London, England.
161. **Fuhrmann, G. F., and A. Rothstein.** 1968. The mechanism of the partial inhibition of fermentation in yeast by nickel ions. Biochim. Biophys. Acta 163:331–338.
162. **Fuller, S. J.** 1991. Biocide-induced enzyme inhibition. Soc. Appl. Bacteriol. Tech. Ser. 27:235–249.
163. **Furr, J. R.** Sensitivity of protozoa to disinfection B. Acanthamoeba and contact lens solutions. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
164. **Furr, J. R., A. D. Russell, T. D. Turner, and A. Andrews.** 1994. Antibacterial activity of Actisorb Plus, Actisorb and silver nitrate. J. Hosp. Infect. 27:201–208.
165. **Gale, E. F.** 1986. Nature and development of phenotypic resistance to amphotericin B in *Candida albicans*. Adv. Microb. Physiol. 27:277–320.
166. **Gandhi, P. A., A. D. Sawant, L. A. Wilson, and D. G. Ahearn.** 1993. Adaptation and growth of *Serratia mascescens* in contact lens disinfectant solutions containing chlorhexidine gluconate. Appl. Environ. Microbiol. 59:183–188.
167. **Gardner, J. F., and K. G. Gray.** 1991. Chlorhexidine, p. 251–270. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
168. **George, A. M.** 1996. Multidrug resistance in enteric and other Gram-negative bacteria. FEMS Microbiol. Lett. 139:1–10.
169. **Gilbert, P.** 1988. Microbial resistance to preservative systems, p. 171–194. *In* S. F. Bloomfield, R. Baird, R. E. Leak, and R. Leech (ed.), Microbial quality assurance in pharmaceuticals, cosmetics and toiletries. Ellis Horwood, Chichester, England.
170. **Gilbert, P., J. Barber, and J. Ford.** 1991. Interaction of biocides with model membranes and isolated membrane fragments. Soc. Appl. Bacteriol. Tech. Ser. 27:155–170.
171. **Gilbert, P., and M. R. W. Brown.** 1995. Some perspectives on preservation and disinfection in the present day. Int. Biodeterior. Biodegrad. 36:219–226.
172. **Gilbert, P., P. J. Collier, and M. R. W. Brown.** 1990. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy and stringent response. Antimicrob. Agents Chemother. 34:1865–1868.
173. **Gilbert, P., D. Pemberton, and D. E. Wilkinson.** 1990. Barrier properties of the Gram-negative cell envelope towards high molecular weight polyhexamethylene biguanides. J. Appl. Bacteriol. 69:585–592.
174. **Gilbert, P., D. Pemberton, and D. E. Wilkinson.** 1990. Synergism within polyhexamethylene biguanide biocide formulations. J. Appl. Bacteriol. 69:593–598.
175. **Gilleland, H. E., Jr., J. D. Stinnett, and R. G. Eagon.** 1974. Ultrastructural and chemical alteration of the cell envelope of *Pseudomonas aeruginosa*, associated with resistance to ethylenediamine tetraacetate resulting from growth in a Mg^{2+}-deficient medium. J. Bacteriol. 117:302–311.
176. **Gomez, R. F., and A. A. Herrero.** 1983. Chemical preservation of foods, p. 77–116. *In* A. H. Rose (ed.), Food microbiology, vol. 8. Economic microbiology. Academic Press, Ltd., London, England.
177. **Gordon, S., and P. W. Andrew.** 1996. Mycobacterial virulence factors. J. Appl. Bacteriol. Symp. Suppl. 81:10S–22S.
178. **Gorman, S. P.** 1991. Microbial adherence and biofilm production. Soc. Appl. Bacteriol. Tech. Ser. 27:271–295.
179. **Gorman, S. P., and E. M. Scott.** 1977. Uptake and media reactivity of glutaraldehyde solutions related to structure and biocidal activity. Microbios Lett. 5:163–169.
180. **Gorman, S. P., E. M. Scott, and E. P. Hutchinson.** 1984. Interaction of the *Bacillus subtilis* spore protoplast, cortex, ion-exchange and coatless forms with glutaraldehyde. J. Appl. Bacteriol. 56:95–102.
181. **Gorman, S. P., E. M. Scott, and E. P. Hutchinson.** 1984. Emergence and development of resistance to antimicrobial chemicals and heat in spores of *Bacillus subtilis*. J. Appl. Bacteriol. 57:153–163.
182. **Gorman, S. P., E. M. Scott, and A. D. Russell.** 1980. Antimicrobial activity, uses and mechanism of action of glutaraldehyde. J. Appl. Bacteriol. 48:161–190.
183. **Gottardi, W.** 1985. The influence of the chemical behavior of iodine on the germicidal action of disinfectant solutions containing iodine. J. Hosp. Infect. 6(Suppl. A):1–11.
184. **Gottardi, W.** 1991. Iodine and iodine compounds, p. 152–166. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation. 4th ed. Lea & Febiger, Philadelphia, Pa.
185. **Grant, K. A., and S. F. Park.** 1995. Molecular characterization of *katA* from *Campylobacter jejuni* and generation of a catalase-deficient mutant of *Campylobacter coli* by interspecific allelic exchange. Microbiology 141:1369–1376.
186. **Gray, T. B., R. T. M. Curson, J. F. Sherwan, and P. R. Rose.** 1995. Acanthamoeba, bacterial and fungal contamination of contact lens storage cases. Br. J. Ophthalmol. 79:601–605.
187. **Griffits, P. A., J. R. Babb, C. R. Bradley, and A. P. Fraise.** 1997. Glutaraldehyde-resistant *Mycobacterium chelonae* from endoscope washer disinfectants. J. Appl. Microbiol. 82:519–526.
188. **Grinius, L., G. Dreguniene, E. B. Goldberg, C.-H. Liao, and S. J. Projan.** 1992. A staphylococcal multidrug resistance gene product is a member of a new protein family. Plasmid 27:119–129.
189. **Grossgebauer, K.** 1970. Virus disinfection, p. 103–148. *In* M. A. Benarde (ed.), Disinfection. Marcel Dekker, Inc., New York, N.Y.
190. **Grossman, L., S. S. Levine, and W. S. Allison.** 1961. The reaction of formaldehyde with nucleotides and T2 bacteriophage DNA. J. Mol. Biol. 3:47–60.
191. **Gump, W. S.** 1977. The bis-phenols, p. 252–281. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
192. **Haefeli, C., C. Franklin, and K. Hardy.** 1984. Plasmid-determined silver resistance in *Pseudomonas stutzeri* isolated from a silver mine. J. Bacteriol. 158:389–392.
193. **Hall, E., and R. G. Eagon.** 1985. Evidence for plasmid-mediated resistance of *Pseudomonas putida* to hexahydro-1,3,5-triethyl-*s*-triazine. Curr. Microbiol. 12:17–22.
194. **Hamilton, W. A.** 1971. Membrane-active anti-bacterial compounds, p. 77–106. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Ltd., London, England.
195. **Hammond, S. A., J. R. Morgan, and A. D. Russell.** 1987. Comparative susceptibility of hospital isolates of Gram-negative bacteria to antiseptics and disinfectants. J. Hosp. Infect. 9:255–264.
196. **Hammond, S. M., P. A. Lambert, and A. N. Rycroft.** 1984. The bacterial cell surface. Croom Helm, London, England.
197. **Hancock, R. E. W.** 1984. Alterations in membrane permeability. Annu. Rev. Microbiol. 38:237–264.
198. **Harakeh, S.** 1987. Inactivation of enteroviruses, rotaviruses, bacteriophages by peracetic acid in a municipal sewage effluent. FEMS Microbiol. Lett. 23:27–30.
199. **Harold, F. M., J. R. Baarda, C. Baron, and A. Abrams.** 1969. Dio 9 and chlorhexidine. Inhibition of membrane bound ATPase and of cation transport in *Streptococcus faecalis*. Biochim. Biophys. Acta 183:129–136.
200. **Hartford, O. M., and B. C. Dowds.** 1994. Isolation and characterization of a hydrogen peroxide resistant mutant of *Bacillus subtilis*. Microbiology 140:297–304.
201. **Hector, R. F.** 1993. Compounds active against cell walls of medically important fungi. Clin. Microbiol. Rev. 6:1–21.
202. **Heinzel, M.** 1988. The phenomena of resistance to disinfectants and preservatives, p. 52–67. *In* K. R. Payne (ed.), Industrial biocides. John Wiley & Sons Ltd., Chichester, England.
203. **Heir, E., G. Sundheim, and A. L. Holck.** 1995. Resistance to quaternary ammonium compounds in *Staphylococcus* spp. isolated from the food in-

dustry and nucleotide sequence of the resistance plasmid pST827. J. Appl. Bacteriol. 79:149–156.
204. **Hiom, S. J., J. R. Furr, A. D. Russell, and J. R. Dickinson.** 1992. Effects of chlorhexidine diacetate on *Candida albicans*, *C. glabrata* and *Saccharomyces cerevisiae*. J. Appl. Bacteriol. 72:335–340.
205. **Hiom, S. J., J. R. Furr, A. D. Russell, and J. R. Dickinson.** 1993. Effects of chlorhexidine diacetate and cetylpyridinium chloride on whole cells and protoplasts of *Saccharomyces cerevisiae*. Microbios 74:111–120.
206. **Hiom, S. J., J. R. Furr, and A. D. Russell.** 1995. Uptake of ^{14}C-chlorohexidine diacetate on by *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. Lett. Appl. Microbiol. 21:20–22.
207. **Hiom, S. J., A. C. Hann, J. R. Furr, and A. D. Russell.** 1995. X-ray microanalysis chlorhexidine-treated cells of *Saccharomyces cerevisiae*. Lett. Appl. Microbiol. 20:353–356.
208. **Hiom, S. J., J. R. Furr, A. D. Russell, and A. C. Hann.** 1996. The possible role of yeast cell walls in modifying cellular response to chlorhexidine diacetate. Cytobios 86:123–135.
209. **Hiraishi, A., K. Furunata, A. Matsumoto, K. A. Koike, M. Fukuyama, and K. Tabuchi.** 1995. Phenotypic and genetic diversity of chlorine-resistant *Methylobacterium* strains isolated from various environments. Appl. Environ. Microbiol. 61:2099–2107.
210. **Hodges, N. A., and G. W. Hanlon.** 1991. Detection and measurement of combined biocide action. Soc. Appl. Bacteriol. Tech. Ser. 27:297–310.
211. **Holton, J., P. Nye, and V. McDonald.** 1994. Efficacy of selected disinfectants vs. Mycobacteria and Cryptosporidium. J. Hosp. Infect. 27:105–115.
212. **Hughes, R. C., and P. F. Thurman.** 1970. Cross-linking of bacterial cell walls with glutaraldehyde. Biochem. J. 119:925–926.
213. **Hugo, W. B.** 1971. Diamidines, p. 121–136. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Ltd., London, England.
214. **Hugo, W. B.** 1991. The degradation of preservatives by micro-organisms. Int. Biodeterior. Biodegrad. 27:185–194.
215. **Hugo, W. B.** Disinfection mechanisms. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
216. **Hugo, W. B., and S. F. Bloomfield.** 1971. Studies on the mode of action of the phenolic antibacterial agent Fentichlor against *Staphylococcus aureus* and *Escherichia coli*. II. The effects of Fentichlor on the bacterial membrane and the cytoplasmic constituents of the cell. J. Appl. Bacteriol. 34:569–578.
217. **Hugo, W. B., and S. F. Bloomfield.** 1971. Studies on the mode of action of the phenolic antibacterial agent Fentichlor against *Staphylococcus aureus* and *Escherichia coli*. III. The effect of Fentichlor on the metabolic activities of *Staphylococcus aureus* and *Escherichia coli*. J. Appl. Bacteriol. 34:579–591.
218. **Hugo, W. B., and J. R. Davidson.** 1973. Effect of cell lipid depletion in *Staphylococcus aureus* upon its resistance to antimicrobial agents. II. A comparison of the response of normal and lipid depleted cells of *S. aureus* to antibacterial drugs. Microbios 8:63–72.
219. **Hugo, W. B., and S. P. Denyer.** 1987. The concentration exponent of disinfectant and preservatives (biocides). Soc. Appl. Bacteriol. Tech. Ser. 22:281–291.
220. **Hugo, W. B., and I. Franklin.** 1968. Cellular lipid and the antistaphylococcal activity of phenols. J. Gen. Microbiol. 52:365–373.
221. **Hugo, W. B., and M. Frier.** 1969. Mode of action of the antibacterial compound dequalinium acetate. Appl. Microbiol. 17:118–127.
222. **Hugo, W. B., and A. R. Longworth.** 1964. Some aspects of the mode of action of chlorhexidine. J. Pharm. Pharmacol. 16:655–662.
223. **Hugo, W. B., and A. R. Longworth.** 1965. Cytological aspects of the mode of action of chlorhexidine. J. Pharm. Pharmacol. 17:28–32.
224. **Hugo, W. B., and A. R. Longworth.** 1966. The effect of chlorhexidine on the electrophoretic mobility, cytoplasmic content, dehydrogenase activity and cell walls of *Escherichia coli* and *Staphylococcus aureus*. J. Pharm. Pharmacol. 18:569–578.
225. **Hugo, W. B., L. J. Pallent, D. J. W. Grant, S. P. Denyer, and A. Davies.** 1986. Factors contributing to the survival of a strain of *Pseudomonas cepacia* in chlorhexidine solutions. Lett. Appl. Microbiol. 2:37–42.
226. **Hugo, W. B., and A. D. Russell.** Types of antimicrobial agents. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
227. **Ikeda, T., S. Tazuke, C. H. Bamford, and A. Ledwith.** 1984. Interaction of a polymeric biguanide with phospholipid membranes. Biochim. Biophys. Acta 769:57–66.
228. **Inderlied, C. B., C. A. Kemper, and L. E. M. Bermudez.** 1993. The *Mycobacterium avium* complex. Clin. Microbiol. Rev. 6:266–310.
229. **Irizarry, L., T. Merlin, J. Rupp, and J. Griffith.** 1996. Reduced susceptibility of methicillin-resistant *Staphylococcus aureus* to cetylpyridinium chloride and chlorhexidine. Chemotherapy 42:248–252.
230. **Ismaeel, N., T. El-Moug, J. R. Furr, and A. D. Russell.** 1986. Resistance of *Providencia stuartii* to chlorhexidine: a consideration of the role of the inner membrane. J. Appl. Bacteriol. 60:361–367.
231. **Izatt, R. M., J. J. Christensen, and J. H. Rytting.** 1971. Sites and thermodynamic quantities associated with proton and metal interaction with ribonucleic acid, deoxyribonucleic acid and their constituent bases, nucleosides and nucleotides. Chem. Rev. 71:439–471.
232. **Jarlier, V., and H. Nikaido.** 1990. Permeability barrier to hydrophilic solutes in *Mycobacterium chelonei*. J. Bacteriol. 172:1418–1423.
233. **Jarroll, E. L.** 1988. Effect of disinfectant on *Giarda* cysts. Crit. Rev. Environ. Control 18:1–28.
234. **Jarroll, E. L.** Sensitivity of protozoa to disinfection. A. Intestinal protozoa. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
235. **Järvinen, H. J. Temovuo, and P. Huovinen.** 1993. In vitro susceptibility of *Streptococcus mutans* to chlorhexidine and six other antimicrobial agents. Antimicrob. Agents Chemother. 37:1158–1159.
236. **Jenkinson, H. F.** 1981. Germination and resistance defects in spores of a *Bacillus subtilis* mutant lacking a coat polypeptide. J. Gen. Microbiol. 127: 81–91.
237. **Jenkinson, H. F., D. Kay, and J. Mandelstam.** 1980. Temporal dissociation of late events in *Bacillus subtilis* sporulation from expression of genes that determine them. J. Bacteriol. 141:793–805.
238. **Joly, B.** 1995. La résistance microbienne à l'action des antiseptiques et désinfectants, p. 52–65. *In* J. Fleurette, J. Freney, and M.-E. Reverdy (ed.), Antisepsie et désinfection. Editions ESKA, Paris, France.
239. **Jones, I. G., and M. Midgley.** 1985. Expression of a plasmid-borne ethidium resistance determinant from *Staphylococcus aureus* in *Escherichia coli*: evidence for an efflux system. FEMS Microbiol. Lett. 28:355–358.
240. **Jones, M. V., T. M. Herd, and H. J. Christie.** 1989. Resistance of *Pseudomonas aeruginosa* to amphoteric and quaternary ammonium biocides. Microbios 58:49–61.
241. **Joswick, H. L., T. R. Corner, J. N. Silvernale, and P. Gerhardt.** 1971. Antimicrobial actions of hexachlorophane: release of cytoplasmic materials. J. Bacteriol. 108:492–500.
242. **Judis, J.** 1962. Studies on the mode of action of phenolic disinfectants. I. Release of radioactivity from carbon-14-labelled *Escherichia coli*. J. Pharm. Sci. 51:261–265.
243. **Kanazawa, A., T. Ikeda, and T. Endo.** 1995. A novel approach to mode of action of cationic biocides: morphological effect on antibacterial activity. J. Appl. Bacteriol. 78:55–60.
244. **Karabit, M. S., O. T. Juneskans, and P. Ludngren.** 1985. Studies on the evaluation of preservative efficacy. I. The determination of antimicrobial characteristics of phenol. Acta Pharm. Suec. 22:281–290.
245. **Karabit, M. S., O. T. Juneskans, and P. Ludngren.** 1988. Studies on the evaluation of preservative efficacy. III. The determination of antimicrobial characteristics of benzalkonium chloride. Int. J. Pharm. 46:141–147.
246. **Kaulfers, P.-M., H. Karch, and R. Laufs.** 1987. Plasmid-mediated formaldehyde resistance in *Serratia marcescens* and *Escherichia coli*: alterations in the cell surface. Zentbl. Bakteriol. Parasitol. Infektionskr. Hyg. I Abt. Orig. Reihe A 226:239–248.
247. **Kaulfers, P.-M., and A. Masquardt.** 1991. Demonstration of formaldehyde dehydrogenase activity in formaldehyde-resistant *Enterobacteriaceae*. FEMS Microbiol. Lett. 65:335–338.
248. **Kemp, G. K. (Alcide Corporation).** 1998. Personal communication.
249. **Keswick, B. H., T. K. Satterwhite, P. C. Johnson, H. L. DuPont, S. L. Secor, J. A. Bitsura, G. W. Gary, and J. C. Hoff.** 1985. Inactivation of Norwalk virus in drinking water by chlorine. Appl. Environ. Microbiol. 50:261–264.
250. **Khor, S. Y., and M. Jegathesan.** 1983. Heavy metal and disinfectant resistance in clinical isolates of Gram-negative rods. Southeast Asian J. Trop. Med. Public Health 14:199–203.
251. **Khunkitti, W., S. V. Avery, D. Lloyd, J. R. Furr, and A. D. Russell.** 1997. Effects of biocides on *Acanthamoeba castellanii* as measured by flow cytometry and plaque assay. J. Antimicrob. Chemother. 40:227–223.
252. **Khunkitti, W., A. C. Hann, D. Lloyd, J. R. Furr, and A. D. Russell.** Biguanide-induced changes in *Acanthamoeba castellanii*: an electron microscopic study. J. Appl. Microbiol., in press.
253. **Khunkitti, W., D. Lloyd, J. R. Furr, and A. D. Russell.** 1996. The lethal effects of biguanides on cysts and trophozoites of *Acanthamoeba castellanii*. J. Appl. Microbiol. 81:73–77.
254. **Khunkitti, W., D. Lloyd, J. R. Furr, and A. D. Russell.** 1997. Aspects of the mechanisms of action of biguanides on cysts and trophozoites of *Acanthamoeba castellanii*. J. Appl. Microbiol. 82:107–114.
255. **Khunkitti, W., D. Lloyd, J. R. Furr, and A. D. Russell.** *Acanthamoeba castellanii*: growth, encystment, excystment and biocide susceptibility. J. Infect., in press.
256. **Kimbrough, R. D.** 1973. Review of the toxicity of hexachlorophene, including its neurotoxicity. J. Clin. Pharmacol. 13:439–451.
257. **Klein, D., and G. McDonnell.** 1998. Unpublished results.
258. **Klein, M., and A. Deforest.** 1963. Antiviral action of germicides. Soap Chem. Spec. 39:70–72.
259. **Klein, M., and A. Deforest.** 1983. Principles of viral inactivation, p. 422–434. *In* S. S. Block (ed.), Disinfection, sterilization and preservation, 3rd ed. Lea & Febiger, Philadelphia, Pa.
260. **Kobayashi, H., M. Tsuzuki, K. Koshimizu, H. Toyama, N. Yoshihara, T.**

Shikata, K. Abe, K. Mizuno, N. Otomo, and T. Oda. 1984. Susceptibility of hepatitis B virus to disinfectants or heat. J. Clin. Microbiol. 20:214–216.
261. Knott, A. G., and A. D. Russell. 1995. Effects of chlorhexidine gluconate on the development of spores of *Bacillus subtilis*. Lett. Appl. Microbiol. 21: 117–120.
262. Knott, A. G., A. D. Russell, and B. N. Dancer. 1995. Development of resistance to biocides during sporulation of *Bacillus subtilis*. J. Appl. Bacteriol. 79:492–498.
263. Kolawole, D. O. 1984. Resistance mechanisms of mucoid-grown *Staphylococcus aureus* to the antibacterial action of some disinfectants and antiseptics. FEMS Microbiol. Lett. 25:205–209.
264. Korich, D. G., J. R. Mead, M. S. Madore, N. A. Sinclair, and C. R. Sterling. 1990. Effects of ozone, chlorine dioxide, chlorine and monochloramine on *Cryptosporidium parvum* oocyst viability. Appl. Environ. Microbiol. 56: 1423–1428.
265. Kroll, R. G., and G. D. Anagnostopoulos. 1981. Potassium leakage as a lethality index of phenol and the effect of solute and water activity. J. Appl. Bacteriol. 50:139–147.
266. Kroll, R. G., and R. A. Patchett. 1991. Biocide-induced perturbations of cell homeostrasis: intracellular pH, membrane potential and solute transport. Soc. Appl. Bacteriol. Tech. Ser. 27:189–202.
267. Kruse, W. C. 1970. Halogen action on bacteria, viruses and protozoa, p. 113–137. *In* Proceedings of the National Special Conference on Disinfection. ASCE, Amherst, Mass.
268. Kulikovsky, A., H. S. Pankratz, and H. L. Sadoff. 1975. Ultrastructural and chemical changes in spores of *Bacillus cereus* after action of disinfectants. J. Appl. Bacteriol. 38:39–46.
269. Kummerle, N., H. H. Feucht, and P. M. Kaulfers. 1996. Plasmid-mediated formaldehyde resistance in *Escherichia coli*: characterization of resistance gene. Antimicrob. Agents Chemother. 40:2276–2279.
270. Kushner, D. J., and S. R. Khan. 1968. Proflavine uptake and release in sensitive and resistant *Escherichia coli*. J. Bacteriol. 96:1103–1114.
271. Kuykendall, J. R., and M. S. Bogdanffy. 1992. Efficiency of DNA-histone crosslinking induced by saturated and unsaturated aldehydes in vitro. Mutat. Res. 283:131–136.
272. Kuyyakanond, T., and L. B. Quesnel. 1992. The mechanism of action of chlorhexidine. FEMS Microbiol. Lett. 100:211–216.
273. Lambert, P. A., and S. M. Hammond. 1973. Potassium fluxes. First indications of membrane damage in microorganisms. Biochem. Biophys. Res. Commun. 54:796–799.
274. Langsrud, S., and G. Sundheim. 1997. Factors contributing to the survival of poultry associated *Pseudomonas* spp. exposed to a quaternary ammonium compound. J. Appl. Microbiol. 82:705–712.
275. Lannigan, R., and L. E. Bryan. 1985. Decreased susceptibility of *Serratia mascescens* to chlorhexidine related to the inner membrane. J. Antimicrob. Chemother. 15:559–565.
276. Lappin-Scott, H. M., and J. W. Costerton. 1995. Microbial biofilms. Cambridge University Press, Cambridge, England.
277. Larson, E. L. 1996. Antiseptics, p. 19-1–19-7, G1–G17. *In* R. N. Olmstad (ed.), APIC infection control & applied epidemiology: principles & practices. Mosby-Year Book, Inc., St. Louis, Mo.
278. Larson, E. L., and H. E. Morton. 1991. Alcohols, p. 191–203. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
279. LeChevalier, M. W., C. C. Cawthorn, and R. G. Lee. 1988. Mechanisms of bacterial survival in chlorinated water supplies. Appl. Environ. Microbiol. 54:2492–2499.
280. Leelaporn, A., N. Firth, I. T. Paulsen, and R. A. Skurray. 1996. IS*257*-mediated cointegration in the evolution of a family of staphylococcal trimethoprim resistance plasmids. J. Bacteriol. 178:6070–6073.
281. Leelaporn, A., I. T. Paulsen, J. M. Tennent, T. G. Littlejohn, and R. A. Skurray. 1994. Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. J. Med. Microbiol. 40:214–220.
282. Leive, L. 1974. The barrier function of the Gram-negative envelope. Ann. N. Y. Acad Sci. 235:109–129.
283. Lensing, H. H., and H. L. Oei. 1984. Study of the efficiency of disinfectants against antrax spores. Tijdschr. Diergeneeskd. 109:557–563.
284. Levy, S. B. 1992. Active efflux mechanisms for antimicrobial resistance. Antimicrob. Agents Chemother. 36:695–703.
285. Lewis, R. 1988. Antiseptic resistance in JK and other coryneforms. J. Hosp. Infect. 11:150–154.
286. Leyval, C., C. Arz, J. C. Block, and M. Rizet. 1984. *Escherichia coli* resistance to chlorine after successive chlorinations. Environ. Technol. Lett. 5: 359–364.
287. Liau, S. Y., D. C. Read, W. J. Pugh, J. R. Furr, and A. D. Russell. 1997. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. Lett. Appl. Microbiol. 25:279–283.
288. Littlejohn, T. G., D. DiBeradino, L. J. Messerotti, S. J. Spiers, and R. A. Skurray. 1990. Structure and evolution of a family of genes encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. Gene 101: 59–66.
289. Littlejohn, T. G., I. T. Paulsen, M. T. Gillespie, J. M. Tennent, M. Midgley, I. G. Jones, A. S. Purewal, and R. A. Skurray. 1992. Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. FEMS Microbiol. Lett. 95:259–266.
290. Longworth, A. R. 1971. Chlorhexidine, p. 95–106. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Ltd., London, England.
291. Loveless, A. 1951. Quality aspects of the chemistry and biology of radiomimetic (mutagenic) substances. Nature (London) 167:338–342.
292. Lukens, R. J. 1983. Chemistry of fungicidal action. Mol. Biol. Biochem. Biophys. 10.
293. Luria, S. E. 1947. Reactivation of irradiated bacteriophage by transfer of self-reproducing units. Proc. Natl. Acad. Sci. USA 33:253.
294. Lynam, P. A., J. R. Babb, and A. P. Fraise. 1995. Comparison of the mycobactericidal activity of 2% alkaline glutaraldehyde and 'Nu-Cidex' (0.35% peracetic acid). J. Hosp. Infect. 30:237–239.
295. Lyon, B. R., and R. A. Skurray. 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiol. Rev. 51:88–134.
296. Lyr, H. 1987. Selectivity in modern fungicides and its basis, p. 31–58. *In* H. Lyr (ed.), Modern selective fungicides. Longman, Harlow, England.
297. Ma, T.-H., and M. M. Harris. 1988. Review of the genotoxicity of formaldehyde. Mutat. Res. 196:37–59.
298. Maillard, J.-Y. 1998. Mechanisms of viricidal action. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
299. Maillard, J.-Y., T. S. Beggs, M. J. Day, R. A. Hudson, and A. D. Russell. 1993. Effect of biocides on *Pseudomonas aeruginosa* phage F116. Lett. Appl. Microbiol. 17:167–170.
300. Maillard, J.-Y., T. S. Beggs, M. J. Day, R. A. Hudson, and A. D. Russell. 1994. Effect of biocides on MS2 and K coliphages. Appl. Environ. Microbiol. 60:2205–2206.
301. Maillard, J.-Y., T. S. Beggs, M. J. Day, R. A. Hudson, and A. D. Russell. 1995. Effects of biocides on the transduction of *Pseudomonas aeruginosa* PAO by F116 bacteriophage. Lett. Appl. Microbiol. 21:215–218.
302. Maillard, J.-Y., T. S. Beggs, M. J. Day, R. Hudson, and A. D. Russell. 1995. Electronmicroscopic investigation of the effects of biocides on *Pseudomonas aeruginosa* PAO bacteriophage F116. J. Med. Microbiol. 42:415–420.
303. Maillard, J.-Y., T. S. Beggs, M. J. Day, R. A. Hudson, and A. D. Russell. 1996. Damage to *Pseudomonas aeruginosa* PAO1 bacteriophage F116 DNA by biocides. J. Appl. Bacteriol. 80:540–554.
304. Maillard, J.-Y., T. S. Beggs, M. J. Day, R. A. Hudson, and A. D. Russell. 1996. The effect of biocides on proteins of *Pseudomonas aeruginosa* PAO bacteriophage F116. J. Appl. Bacteriol. 80:291–295.
305. Maillard, J.-Y., T. S. Beggs, M. J. Day, R. A. Hudson, and A. D. Russell. 1996. The use of an automated assay to assess phage survival after a biocidal treatment. J. Appl. Bacteriol. 80:605–610.
306. Maillard, J.-Y., A. C. Hann, T. S. Beggs, M. J. Day, R. A. Hudson, and A. D. Russell. 1995. Energy dispersive analysis of x-rays study of the distribution of chlorhexidine diacetate and cetylpyridinium chloride on the *Pseudomonas aeruginosa* bacteriophage F116. Lett. Appl. Microbiol. 20:357–360.
307. Maillard, J.-Y., and A. D. Russell. 1997. Viricidal activity and mechanisms of action of biocides. Sci. Progr. 80:287–315.
308. Malchesky, P. S. 1993. Peracetic acid and its application to medical instrument sterilization. Artif. Organs 17:147–152.
309. Manuelidis, L. 1997. Decontamination of Creutzfeldt-Jakob disease and other transmissible agents. J. Neurovirol. 3:62–65.
310. Marrie, T. J., and J. W. Costerton. 1981. Prolonged survival of *Serratia marcescens* in chlorhexidine. Appl. Environ. Microbiol. 42:1093–1102.
311. Martin, T. D. M. 1969. Sensitivity of the genus *Proteus* to chlorhexidine. J. Med. Microbiol. 2:101–108.
312. Martindale Extra Pharmacopoeia. 1993. Silver nitrate, p. 1412; silver sulfadiazine, p. 201. Pharmaceutical Press, London, England.
313. Marzulli, F. N., and M. Bruch. 1981. Antimicrobial soaps: benefits versus risks, p. 125–134. *In* H. Maibach and R. Aly (ed.), Skin microbiology: relevance to clinical infection. Springer-Verlag, New York, N.Y.
314. Mayworm, D. 1998. Low temperature sterilization revisited. Infect. Control Steril. Tech. 4:18–35.
315. Mbithi, J. N., V. S. Springthorpe, and S. A. Sattar. 1990. Chemical disinfection of hepatitis: a virus on environmental surfaces. Appl. Environ. Microbiol. 56:3601–3604.
316. Mbithi, J. N., V. S. Springthorpe, S. A. Sattar, and M. Pacquette. 1993. Bactericidal, virucidal, and mycobactericidal activities of reused alkaline gluteraldehyde in an endoscopy unit. J. Clin. Microbiol. 31:2988–2995.
317. McClure, A. R., and J. Gordon. 1992. *In vitro* evaluation of povidone-iodine and chlorhexidine against methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. 21:291–299.
318. McDonnell, G. 1998. Unpublished results.
319. McDonnell, G., K. Kornberger, and D. Pretzer. 1997. Antiseptic resistance: a survey of *Staphylococcus* and *Enterococcus*. *In* The healthcare continuum model: topical antimicrobial wash products in healthcare settings, the food industry and the home, June 1997. SDA/CTFA, Washington, D.C.
320. McGucken, P. V., and W. Woodside. 1973. Studies on the mode of action of

glutaraldehyde on *Escherichia coli*. J. Appl. Bacteriol. 36:419–426.
321. **McKenna, S. M., and K. J. A. Davies.** 1988. The inhibition of bacterial growth by hypochlorous acid. Biochem. J. 254:685–692.
322. **McNeil, M. R., and P. J. Brennan.** 1991. Structure, function and biogenesis of the cell envelope of mycobacteria in relation to bacterial physiology, pathogenesis and drug resistance: some thoughts and possibilities arising from recent structural information. Res. Microbiol. 142:451–463.
323. **Michele, T. M., W. A. Cronin, N. M. H. Graham, D. M. Dwyer, D. S. Pope, S. Harrington, R. E. Chaisson, and W. R. Bishai.** 1997. Transmission of *Mycobacterium tuberculosis* by a fiberoptic bronchoscope. JAMA 278:1093–1095.
324. **Midgley, M.** 1986. The phosphonium ion ion efflux system of *Escherichia coli*: a relationship to the ethidium efflux system and energetic studies. J. Gen. Microbiol. 132:3187–3193.
325. **Midgley, M.** 1987. An efflux system for cationic dyes and related compounds in *Escherichia coli*. Microbiol. Sci. 14:125–127.
326. **Midgley, M.** 1994. Characteristics of an ethidium efflux system in *Enterococcus hirae*. FEMS Microbiol. Lett. 120:119–124.
327. **Milhaud, P., and G. Balassa.** 1973. Biochemical genetics of bacterial sporulation. IV. Sequential development of resistance to chemical and physical agents during sporulation of *Bacillus subtilis*. Mol. Gen. Genet. 125:241–250.
328. **Miller, L. P.** 1969. Mechanisms for reaching the site of actin, p. 1–58. *In* D. C. Torgeson (ed.), Fungicides: an advanced treatise, vol. 2. Academic Press, Inc., New York, N.Y.
329. **Miller, L. P., and S. E. A. McCallan.** 1957. Toxic action of metal ions to fungus spores. Agric. Food Chem. 5:116–122.
330. **Miller, P. F., and M. C. Sulavik.** 1996. Overlaps and parallels in the regulation of intrinsic multiple-antibiotic resistance in *Escherichia coli*. Mol. Microbiol. 21:441–448.
331. **Misra, T. K.** 1992. Bacterial resistance to organic mercury salts and organomercurials. Plasmid 27:17–28.
332. **Modak, S. M., and C. L. Fox, Jr.** 1973. Binding of silver sulfadiazine to the cellular components of *Pseudomonas aeruginosa*. Biochem. Pharmacol. 22: 2391–2404.
333. **Moken, M. C., L. M. McMurry, and S. B. Levy.** 1997. Selection of multiple-antibiotic-resistant (Mar) mutants of *Escherichia coli* by using the disinfectant pine oil: roles of the *mar* and *acrAB* loci. Antimicrob. Agents Chemother. 41:2770–2772.
334. **Moore, F. C., and L. R. Perkinson.** 1979. U.S. patent 4,169,123.
335. **Morgan, R. W., M. F. Christman, F. S. Jacobson, G. Storz, and B. N. Ames.** 1986. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA 83:8059–8063.
336. **Morris, J. G., Jr., M. B. Sztein, E. W. Rice, J. P. Nataro, G. A. Losonsky, P. Panigrahi, C. O. Tacket, and J. A. Johnson.** 1996. *Vibrio cholerae* O1 can assume a chlorine-resistant rugose survival form that is virulent for humans. J. Infect. Dis. 174:1364–1368.
337. **Morton, H. E.** 1983. Alcohols, p. 225–239. *In* S. S. Bloch (ed.), Disinfection, sterilization, and preservation, 3rd ed. Lea & Febiger, Philadelphia, Pa.
338. **Mukhopadhyay, S., and H. E. Schellhorn.** 1997. Identification and characterization of hydrogen peroxide-sensitive mutants of *Escherichia coli*: genes that require OxyR for expression. J. Bacteriol. 179:330–338.
339. **Munton, T. J., and A. D. Russell.** 1970. Aspects of the action of glutaraldehyde on *Escherichia coli*. J. Appl. Bacteriol. 33:410–419.
340. **Munton, T. J., and A. D. Russell.** 1970. Effect of glutaraldehyde on protoplasts of *Bacillus megaterium*. J. Gen. Microbiol. 63:367–370.
341. **Munton, T. J., and A. D. Russell.** 1971. Interaction of glutaraldehyde with some micro-organisms. Experientia 27:109–110.
342. **Munton, T. J., and A. D. Russell.** 1972. Effect of glutaraldehyde on the outer layers of *Escherichia coli*. J. Appl. Bacteriol. 35:193–199.
343. **Munton, T. J., and A. D. Russell.** 1973. Effect of glutaraldehyde on cell viability, triphenyltetrazolium reduction, oxygen uptake and β-galactosidase activity in *Escherichia coli*. Appl. Microbiol. 26:508–511.
344. **Munton, T. J., and A. D. Russell.** 1973. Interaction of glutaraldehyde with spheroplasts of *Escherichia coli*. J. Appl. Bacteriol. 36:211–217.
345. **Musser, J. M.** 1995. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin. Microbiol. Rev. 8:496–514.
346. **Mycock, G.** 1985. Methicillin/antiseptic-resistant *Staphylococcus aureus*. Lancet ii:949–950.
347. **Myers, J. A., M. C. Allwood, M. J. Gidley, and J. K. M. Sanders.** 1980. The relationship between structure and activity of taurolin. J. Appl. Bacteriol. 48:89–96.
348. **Nagai, I., and H. Ogase.** 1990. Absence of role for plasmids in resistance to multiple disinfectants in three strains of bacteria. J. Hosp. Infect. 15:149–155.
349. **Nies, D. H., and S. Silver.** 1995. Ion efflux systems involved in bacterial metal resistances. J. Ind. Microbiol. 14:186–199.
350. **Nakajima, H., K. Kobayashi, M. Kobayashi, H. Asako, and R. Aono.** 1995. Overexpression of the *robA* gene increases organic solvent tolerance and multiple antibiotic and heavy metal ion resistance in *Escherichia coli*. Appl. Environ. Microbiol. 61:2302–2307.
351. **Nakamura, H.** 1966. Acriflavine-binding capacity of *Escherichia coli* in relation to acriflavine sensitivity and metabolic activity. J. Bacteriol. 92: 1447–1452.
352. **Navarro, J. M., and P. Monsan.** 1976. Étude du mécanisme d'interaction du glutaraldéhyde avec les microorganismes. Ann. Microbiol. (Inst. Pasteur) 127B:295–307.
353. **Nicholson, G., R. A. Hudson, M. V. Chadwick, and H. Gaya.** 1995. The efficacy of the disinfection of bronchoscopes contaminated in vitro with *Mycobacterium tuberculosis* and *Mycobacterium avium intracellulose* in sputum: a comparison of Sactimed-I-Sinald and glutaraldehyde. J. Hosp. Infect. 29:257–264.
354. **Nicoletti, G., V. Boghossian, F. Gureviteh, R. Borland, and P. Morgenroth.** 1993. The antimicrobial activity *in vitro* of chlorhexidine, a mixture of isothiazolinones ('Kathon' CG) and cetyltrimethylammonium bromide (CTAB). J. Hosp. Infect. 23:87–111.
355. **Nikaido, H.** 1994. Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 264:382–388.
356. **Nikaido, H., S.-H. Kim, and E. Y. Rosenberg.** 1993. Physical organization of lipids in the cell wall of *Mycobacterium chelonae*. Mol. Microbiol. 8:1025–1030.
357. **O'Brien, R. T., and J. Newman.** 1979. Structural and compositional changes associated with chlorine inactivation of polioviruses. Appl. Environ. Microbiol. 38:1034–1039.
358. **Ogase, H., I. Nigai, K. Kameda, S. Kume, and S. Ono.** 1992. Identification and quantitative analysis of degradation products of chlorhexidine with chlorhexidine-resistant bacteria with three-dimensional high performance liquid chromatography. J. Appl. Bacteriol. 73:71–78.
359. **Olivieri, V. P., C. W. Kruse, Y. C. Hsu, A. C. Griffiths, and K. Kawata.** 1975. The comparative mode of action of chlorine, bromine, and iodine of f2 bacterial virus, p. 145–162. *In* J. D. Johnson (ed.), Disinfection-water and wastewater. Ann Arbor Science, Ann Arbor, Mich.
360. **Pallent, L. J., W. B. Hugo, D. J. W. Grant, and A. Davies.** 1983. *Pseudomonas cepacia* and infections. J. Hosp. Infect. 4:9–13.
361. **Park, J. B., and N. H. Park.** 1989. Effect of chlorhexidine on the *in vitro* and *in vivo* herpes simplex virus infection. Oral Surg. 67:149–153.
362. **Passagot, J., J. M. Crance, E. Biziagos, H. Laveran, F. Agbalika, and R. Deloince.** 1987. Effect of glutaraldehyde on the antigenicity and infectivity of hepatitis A virus. J. Virol. Methods 16:21–28.
363. **Paulsen, I. T., M. H. Brown, S. J. Dunstan, and R. A. Skurray.** 1995. Molecular characterization of the staphylococcal multidrug resistance export protein QacC. J. Bacteriol. 177:2827–2833.
364. **Paulsen, I. T., M. H. Brown, T. G. Littlejohn, B. A. Mitchell, and R. A. Skurray.** 1996. Multidrug resistance proteins QacA and QacB from *Staphylococcus aureus*: membrane topology and identification of residues involved in substrate specificity. Proc. Natl. Acad. Sci. USA 93:3630–3635.
365. **Paulsen, I. T., T. G. Littlejohn, P. Radstrom, L. Sundstrom, O. Skold, G. Swedberg, and R. A. Skurray.** 1993. The 31 conserved segment of integrons contain a gene associated with multidrug resistance to antiseptics and disinfectants. Antimicrob. Agents Chemother. 34:761–768.
366. **Paulsen, I. T., J. H. Park, P. S. Choi, and M. H. Saier.** 1997. A family of gram-negative outer membrane factors that function in the export of proteins, carbohydrates, drugs and heavy metals from gram-negative bacteria. FEMS Microbiol. Lett. 156:1–8.
367. **Paulsen, I. T., R. A. Skurray, R. Tam, M. H. Saier, R. J. Turner, J. H. Weiner, E. B. Goldberg, and L. L. Grinius.** 1996. The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol. Microbiol. 19:1167–1175.
368. **Paulsen, I. T., and R. A. Skurray.** 1993. Topology, structure and evolution of two families of proteins involved in antibiotic and antiseptic resistance in eukaryotes and prokaryotes—an analysis. Gene 124:1–11.
369. **Permana, P. A., and R. M. Snapka.** 1994. Aldehyde-induced protein-DNA crosslinks disrupt specific stages of SV40 DNA replication. Carcinogenesis 15:1031–1036.
370. **Persino, R., and D. L. Lynch.** 1982. Divalent cation dependent resistance in *E. coli* LMR-26 to the broad spectrum antimicrobial agent Irgasan. Microbios 34:41–58.
371. **Phillips, C. R.** 1952. Relative resistance of bacterial spores and vegetative bacteria to disinfectants. Bacteriol. Rev. 16:135–138.
372. **Phillips-Jones, M. K., and M. E. Rhodes-Roberts.** 1991. Studies of inhibitors of respiratory electron transport and oxidative phosphorylation. Soc. Appl. Bacteriol. Tech. Ser. 27:203–224.
373. **Pitt, T. L., M. Gaston, and P. N. Hoffman.** 1983. In vitro susceptibility of hospital isolates in various bacterial genera to chlorhexidine. J. Hosp. Infect. 4:173–176.
374. **Power, E. G. M.** 1995. Aldehydes as biocides. Prog. Med. Chem. 34:149–201.
375. **Power, E. G. M., B. N. Dancer, and A. D. Russell.** 1988. Emergence of resistance to glutaraldehyde in spores of *Bacillus subtilis* 168. FEMS Microbiol. Lett. 50:223–226.
376. **Power, E. G. M., B. N. Dancer, and A. D. Russell.** 1989. Possible mechanisms for the revival of glutaraldehyde-treated spores of *Bacillus subtilis* NCTC 8236. J. Appl. Bacteriol. 67:91–98.

377. **Power, E. G. M., B. N. Dancer, and A. D. Russell.** 1990. Effect of sodium hydroxide and two proteases on the revival of aldehyde-treated spores of *Bacillus subtilis*. Lett. Appl. Microbiol. **10**:9–13.
378. **Power, E. G. M., and A. D. Russell.** 1989. Glutaraldehyde: its uptake by sporing and non-sporing bacteria, rubber, plastic and an endoscope. J. Appl. Bacteriol. **67**:329–342.
379. **Power, E. G. M., and A. D. Russell.** 1989. Uptake of L-(^{14}C)-alanine to glutaraldehyde-treated and untreated spores of *Bacillus subtilis*. FEMS Microbiol. Lett. **66**:271–276.
380. **Power, E. G. M., and A. D. Russell.** 1990. Sporicidal action of alkaline glutaraldehyde: factors influencing activity and a comparison with other aldehydes. J. Appl. Bacteriol. **69**:261–268.
381. **Poxton, I. R.** 1993. Prokaryote envelope diversity. J. Appl. Bacteriol. Symp. Suppl. **70**:1S–11S.
382. **Prince, D. L., H. N. Prince, O. Thraenhart, E. Muchmore, E. Bonder, and J. Pugh.** 1993. Methodological approaches to disinfection of human hepatitis B virus. J. Clin. Microbiol. **31**:3296–3304.
383. **Prince, H. N., W. S. Nonemaker, R. C. Norgard, and D. L. Prince.** 1978. Drug resistance with topical antiseptics. J. Pharm. Sci. **67**:1629–1631.
384. **Prince, H. N., D. L. Prince, and R. N. Prince.** 1991. Principles of viral control and transmission, p. 411–444. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
385. **Prusiner, S. B.** 1982. Novel proteinaceous infectious particles cause scrapie. Science **216**:136–144.
386. **Pulvertaft, R. J. V., and G. D. Lumb.** 1948. Bacterial lysis and antiseptics. J. Hyg. **46**:62–64.
387. **Rahn, R. O., and L. C. Landry.** 1973. Ultraviolet irradiation of nucleic acids complexed with heavy atoms. II. Phosphorescence and photodimerization of DNA complexed with Ag^+. Photochem. Photobiol. **18**:29–38.
388. **Rahn, R. O., J. K. Setlow, and L. C. Landry.** 1973. Ultraviolet irradiation of nucleic acids complexed with heavy atoms. III. Influence of Ag^+ and Hg^+ on the sensitivity of phage and of transforming DNA to ultraviolet radiation. Photochem. Photobiol. **18**:39–41.
389. **Ranganthan, N. S.** 1996. Chlorhexidine, p. 235–264. *In* J. M. Ascenzi (ed.), Handbook of disinfectants and antiseptics. Marcel Dekker, Inc., New York, N.Y.
390. **Rastogi, N. S., C. Frehel, A. Ryter, H. Ohayon, M. Lesowd, and H. L. David.** 1981. Multiple drug resistance in *Mycobacterium avium*: is the wall architecture responsible for the exclusion of antimicrobial agents? Antimicrob. Agents Chemother. **20**:666–677.
391. **Rastogi, N. S., K. S. Goh, and H. L. David.** 1990. Enhancement of drug susceptibility of *Mycobacterium avium* by inhibitors of cell envelope synthesis. Antimicrob. Agents Chemother. **34**:759–764.
392. **Rayman, M. K., T. C. Y. Lo, and B. D. Sanwal.** 1972. Transport of succinate in *Escherichia coli*. J. Biol. Chem. **247**:6332–6339.
393. **Regos, J., and H. R. Hitz.** 1974. Investigations on the mode of action of triclosan, a broad spectrum antimicrobial agent. Zentbl. Bakteriol. Mikrobiol. Hyg. 1 Abt Orig. **226**:390–401.
394. **Resnick, L., K. Varen, S. Z. Salahuddin, S. Tondreau, and P. D. Markham.** 1986. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. JAMA **255**:1887–1891.
395. **Reverdy, M. E., M. Bes, Y. Brun, and J. Fleurette.** 1993. Évolution de la résistance aux antibiotiques et aux antiseptiques de souche hospitalières de *Staphylococcus aureus* isolées de 1980 à 1991. Pathol. Biol. **41**:897–904.
396. **Reverdy, M.-E., M. Bes, C. Nervi, A. Martra, and J. Fleurette.** 1992. Activity of four antiseptics (acriflavine, benzalkonium chloride, chlorhexidine digluconate and hexamidine di-isethionate) and of ethidium bromide on 392 strains representing 26 *Staphylococcus* species. Med. Microbiol. Lett. **1**:56–63.
397. **Richards, R. M. E.** 1981. Antimicrobial action of silver nitrate. Microbios **31**:83–91.
398. **Richards, R. M. E., H. A. Odelola, and B. Anderson.** 1984. Effect of silver on whole cells and spheroplasts of a silver resistant *Pseudomonas aeruginosa*. Microbios **39**:151–158.
399. **Richards, R. M. E., R. B. Taylor, and D. K. L. Xing.** 1991. An evaluation of the antibacterial activities of sulfonamides, trimethoprim, dibromopropamidine, and silver nitrate compared with their uptakes by selected bacteria. J. Pharm. Sci. **80**:861–867.
400. **Richards, R. M. E., J. Z. Xing, D. W. Gregory, and D. Marshall.** 1993. Investigation of cell envelope damage to *Pseudomonas aeruginosa* and *Enterobacter cloacae* by dibromopropamidine isethionate. J. Pharm. Sci. **82**: 975–977.
401. **Rogers, F. G., P. Hufton, E. Kurzawska, C. Molloy, and S. Morgan.** 1985. Morphological response of human rotavirus to ultraviolet radiation, heat and disinfectants. J. Med. Microbiol. **20**:123–130.
402. **Rose, A. H.** 1987. Responses to the chemical environment, p. 5–40. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, 2nd ed., vol. 2. Yeasts and the environment. Academic Press, Ltd., London, England.
403. **Rosenberg, A., S. D. Alatary, and A. F. Peterson.** 1976. Safety and efficacy of the antiseptic chlorhexidine gluconate. Surg. Gynecol. Obstet. **143**:789–792.
404. **Rosenkranz, H. S., and S. Rosenkranz.** 1972. Silver sulfadiazine: interaction with isolated deoxyribonucleic acid. Antimicrob. Agents Chemother. **2**:373–383.
405. **Rouche, D. A., D. S. Cram, D. Di Berardino, T. G. Littlejohn, and R. A. Skurray.** 1990. Efflux-mediated antiseptic gene *qac*A from *Staphylococcus aureus*: common ancestry with tetracycline and sugar-transport proteins. Mol. Microbiol. **4**:2051–2062.
406. **Roussow, F. T., and R. J. Rowbury.** 1984. Effects of the resistance plasmid R124 on the level of the OmpF outer membrane protein and on the response of *Escherichia coli* to environmental agents. J. Appl. Bacteriol. **56**: 73–79.
407. **Rubin, J.** 1991. Human immunodeficiency virus (HIV) disinfection and control, p. 472–481. *In* S. S. Block (ed.), Disinfection, sterilization and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
408. **Rudolf, A. S., and M. Levine.** 1941. Iowa Engineering and Experimental Station bulletin 150. Iowa Engineering and Experimental Station, Iowa.
409. **Russell, A. D.** 1968. Use of protoplasts, spheroplasts, L-forms and pleuropneumonia-like organisms in disinfection studies. Lab. Pract. **17**:804–808.
410. **Russell, A. D.** 1971. Ethylenediamine tetraacetic acid, p. 209–224. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Ltd., London, England.
411. **Russell, A. D.** 1981. Modification of the bacterial cell envelope and enhancement of antibiotic susceptibility, p. 119–165. *In* C. H. Stuart-Harris and D. M. Harris (ed.), The control of antibiotic-resistant bacteria. Academic Press, Ltd., London, England.
412. **Russell, A. D.** 1982. The destruction of bacterial spores, p. 169–231. Academic Press, Ltd., London, England.
413. **Russell, A. D.** 1985. The role of plasmids in bacterial resistance to antiseptics, disinfectants and preservatives. J. Hosp. Infect. **6**:9–19.
414. **Russell, A. D.** 1990. Bacterial spores and chemical sporicidal agents. Clin. Microbiol. Rev. **3**:99–119.
415. **Russell, A. D.** 1990. Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. J. Appl. Bacteriol. **71**:191–201.
416. **Russell, A. D.** 1991. Chemical sporicidal and sporostatic agents, p. 365–376. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
417. **Russell, A. D.** 1991. Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. J. Appl. Bacteriol. **71**:191–201.
418. **Russell, A. D.** 1992. Effect of liquid phase antibacterial agents, p. 169–231. *In* A. D. Russell, The destruction of bacterial spores. Academic Press, Ltd., London, England.
419. **Russell, A. D.** 1996. Activity of biocides against mycobacteria. J. Appl. Bacteriol, Symp. Suppl. **81**:87S–101S.
420. **Russell, A. D.** 1993. Microbial cell walls and resistance of bacteria to antibiotics and biocides. J. Infect. Dis. **168**:1339–1340.
421. **Russell, A. D.** 1994. Glutaraldehyde: current status and uses. Infect. Control Hosp. Epidemiol. **15**:724–733.
422. **Russell, A. D.** 1995. Mechanisms of bacterial resistance to biocides. Int. Biodeterior. Biodegrad. **36**:247–265.
423. **Russell, A. D.** 1997. Plasmids and bacterial resistance to biocides. J. Appl. Microbiol. **82**:155–165.
424. **Russell, A. D.** Assessment of sporicidal activity. Int Biodeterior. Biodegrad., in press.
425. **Russell, A. D.** Mechanisms of bacterial resistance to antibiotics and biocides. Progr. Med. Chem., in press.
426. **Russell, A. D.** Antifungal activity of biocides. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
427. **Russell, A. D.** Plasmids and bacterial resistance. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
428. **Russell, A. D., and I. Chopra.** 1996. Understanding antibacterial action and resistance, 2nd ed. Ellis Horwood, Chichester, England.
429. **Russell, A. D., B. N. Dancer, and E. G. M. Power.** 1991. Effects of chemical agents on bacterial sporulation, germination and outgrowth. Soc. Appl. Bacteriol. Tech. Ser. **27**:23–44.
430. **Russell, A. D., and M. J. Day.** 1993. Antibacterial activity of chlorhexidine. J. Hosp. Infect. **25**:229–238.
431. **Russell, A. D., and M. J. Day.** 1996. Antibiotic and biocide resistance in bacteria. Microbios **85**:45–65.
432. **Russell, A. D., and J. R. Furr.** 1977. The antibacterial activity of a new chloroxylenol formulation containing ethylenediamine tetraacetic acid. J. Appl. Bacteriol. **43**:253–260.
433. **Russell, A. D., and J. R. Furr.** 1986. The effects of antiseptics, disinfectants and preservatives on smooth, rough and deep rough strains of *Salmonella typhimurium*. Int. J. Pharm. **34**:115–123.
434. **Russell, A. D., and J. R. Furr.** 1986. Susceptibility of some porin- and lipopolysaccharide-deficient strains of *Escherichia coli* to some antiseptics and disinfectants. J. Hosp. Infect. **8**:47–56.
435. **Russell, A. D., and J. R. Furr.** 1987. Comparative sensitivity of smooth,

rough and deep rough strains of *Escherichia coli* to chlorhexidine, quaternary ammonium compounds and dibromopropamidine isethionate. Int. J. Pharm. 36:191–197.
436. **Russell, A. D., and J. R. Furr.** 1996. Biocides: mechanisms of antifungal action and fungal resistance. Sci. Prog. 79:27–48.
437. **Russell, A. D., J. R. Furr, and J.-Y. Maillard.** 1997. Microbial susceptibility and resistance to biocides. ASM News 63:481–487.
438. **Russell, A. D., J. R. Furr, and W. J. Pugh.** 1985. Susceptibility of porin- and lipopolysaccharide-deficient mutants of *Escherichia coli* to a homologous series of esters of *p*-hydroxybenzoic acid. Int. J. Pharm. 27:163–173.
439. **Russell, A. D., J. R. Furr, and W. J. Pugh.** 1987. Sequential loss of outer membrane lipopolysaccharide and sensitivity of *Escherichia coli* to antibacterial agents. Int. J. Pharm. 35:227–233.
440. **Russell, A. D., and G. W. Gould.** 1988. Resistance of Enterobacteriaceae to preservatives and disinfectants. J. Appl. Bacteriol. Symp. Suppl. 65:167S–195S.
441. **Russell, A. D., and H. Haque.** 1975. Inhibition of EDTA-lysozyme lysis of *Pseudomonas aeruginosa* by glutaraldehyde. Microbios 13:151–153.
442. **Russell, A. D., and D. Hopwood.** 1976. The biological uses and importance of glutaraldehyde. Prog. Med. Chem. 13:271–301.
443. **Russell, A. D., and W. B. Hugo.** 1994. Antimicrobial activity and action of silver. Prog. Med. Chem. 31:351–371.
444. **Russell, A. D., and W. B. Hugo.** 1987. Chemical disinfectants, p. 12–42. *In* A. H. Linton, W. B. Hugo, and A. D. Russell (ed.), Disinfection in veterinary and farm animal practice. Blackwell Scientific Publications, Oxford, England.
445. **Russell, A. D., and W. B. Hugo.** 1988. Perturbation of homeostatic mechanisms in bacteria by pharmaceuticals, p. 206–219. *In* R. Whittenbury, G. W. Gould, J. G. Banks, and R. G. Board (ed.), Homeostatic mechanisms in microorganisms. Bath University Press, Bath, England.
446. **Russell, A. D., W. B. Hugo, and G. A. J. Ayliffe (ed.).** 1992. Principle and practices of disinfection, preservation and sterilization, 2nd ed. Blackwell Scientific Publications Ltd., Oxford, England.
447. **Russell, A. D., B. D. Jones, and P. Milburn.** 1985. Reversal of the inhibition of bacterial spore germination and outgrowth by antibacterial agents. Int. J. Pharm. 25:105–112.
448. **Russell, A. D., A. Morris, and M. C. Allwood.** 1973. Methods for assessing damage to bacteria induced by chemical and physical agents. Methods Microbiol. 8:95–182.
449. **Russell, A. D., and A. P. Mills.** 1974. Comparative sensitivity and resistance of some strains of *Pseudomonas aeruginosa* and *Pseudomonas stutzeri* to antibacterial agents. J. Clin. Pathol. 27:463–466.
450. **Russell, A. D., and T. J. Munton.** 1974. Bactericidal and bacteriostatic activity of glutaraldehyde and its interaction with lysine and proteins. Microbios 11:147–152.
451. **Russell, A. D., and N. J. Russell.** 1995. Biocides: activity, action and resistance. Symp. Soc. Gen. Microbiol. 53:327–365.
452. **Russell, A. D., U. Tattawajaet, J.-Y. Maillard, and A. D. Russell.** Possible linked bacterial resistance to antibiotics and biocides. Antimicrob. Agents Chemother, in press.
453. **Russell, A. D., and G. N. Vernon.** 1975. Inhibition by glutaraldehyde of lysostaphin-induced lysis of *Staphylococcus aureus*. Microbios 13:147–149.
454. **Rutala, W. A.** 1995. APIC guidelines for selection and use of disinfectants. Am. J. Infect. Control 23:313–342.
455. **Rutala, W. A., E. C. Cole, M. S. Wannamaker, and D. J. Weber.** 1991. Inactivation of *Mycobacterium tuberculosis* and *Mycobacterium bovis* by 14 hospital disinfectants. Am. J. Med. 91(Suppl. B):267S–271S.
456. **Sabli, M. Z. H., P. Setlow, and W. M. Waites.** 1996. The effect of hypochlorite on spores of *Bacillus subtilis* lacking small acid-soluble proteins. Lett. Appl. Microbiol. 22:405–507.
457. **Sagripanti, J.-L., and A. Bonifacino.** 1996. Comparative sporicidal effects of liquid chemical agents. Appl. Environ. Microbiol. 62:545–551.
458. **Salk, J. E., and J. B. Gori.** 1960. A review of theoretical, experimental and practical considerations in the use of formaldehyde for inactivation of poliovirus. Ann. N. Y. Acad. Sci. 83:609–637.
459. **Salt, W. G., and D. Wiseman.** 1991. Biocide uptake by bacteria. Soc. Appl. Bacteriol. Tech. Ser. 27:65–86.
460. **Salton, M. R. J.** 1968. Lytic agents, cell permeability and monolayer penetrability. J. Gen. Physiol. 52:277S–252S.
461. **Sareen, M., and G. K. Khuller.** 1988. Phospholipids of ethambutol-susceptible and resistant strains of *Mycobacterium smegmatis*. J. Biosci. 13:243–248.
462. **Sareen, M., and G. K. Khuller.** 1990. Cell wall composition of ethambutol-susceptible and resistant strains of *Mycobacterium smegmatis* A7CC607. Lett. Appl. Microbiol. 11:7–10.
463. **Sasatsu, M., Y. Shibata, N. Noguchi, and M. Kono.** 1992. High-level resistance to ethidium bromide and antiseptics in *Staphylococcus aureus*. FEMS Microbiol. Lett. 93:109–114.
464. **Sasatsu, M., Y. Shidata, N. Noguchi, and M. Kono.** 1994. Substrates an inhibitors of antiseptic resistance in *Staphylococcus aureus*. Biol. Pharm. Bull. 17:163–165.
465. **Sasatsu, M., K. Shimuzu, N. Noguchi, and M. Kono.** 1993. Triclosan-resistant *Staphylococcus aureus*. Lancet 341:756.
466. **Sasatsu, M., Y. Shirai, M. Hase, N. Noguchi, M. Kono, H. Behr, J. Freney, and T. Arai.** 1995. The origin of the antiseptic-resistance gene *ebr* in *Staphylococcus aureus*. Microbios 84:161–169.
467. **Sattar, S. A., V. S. Springthorpe, B. Conway, and Y. Xu.** 1994. Inactivation of the human immunodeficiency virus: an update. Rev. Med. Microbiol. 5: 139–150.
468. **Sautter, R. L., L. H. Mattman, and R. C. Legaspi.** 1984. *Serratia marcescens* meningitis associated with a contaminated benzalkonium chloride solution. Infect. Control 5:223–225.
469. **Savage, C. A.** 1971. A new bacteriostat for skin care products. Drug Cosmet. Ind. 109:36–39, 161–163.
470. **Schreurs, W. J. A., and H. Rosenburgh.** 1982. Effect of silver ions on transport and retention of phosphate by *Escherichia coli*. J. Bacteriol. 152:7–13.
471. **Setlow, B., and P. Setlow.** 1993. Binding of small, acid-soluble spore proteins to DNA plays a significant role in the resistance of *Bacillus subtilis* spores to hydrogen peroxide. Appl. Environ. Microbiol. 59:3418–3423.
472. **Setlow, P.** 1994. Mechanisms which contribute to the long-term survival of spores of *Bacillus* species. J. Appl. Bacteriol. Symp. Suppl. 76:49S–60S.
473. **Shaker, L. A., B. N. Dancer, A. D. Russell, and J. R. Furr.** 1988. Emergence and development of chlorhexidine resistance during sporulation of *Bacillus subtilis* 168. FEMS Microbiol. Lett. 51:73–76.
474. **Shaker, L. A., J. R. Furr, and A. D. Russell.** 1988. Mechanism of resistance of *Bacillus subtilis* spores to chlorhexidine. J. Appl. Bacteriol. 64:531–539.
475. **Shaker, L. A., A. D. Russell, and J. R. Furr.** 1986. Aspects of the action of chlorhexidine on bacterial spores. Int. J. Pharm. 34:51–56.
476. **Shields, M. S., M. J. Reagin, R. R. Gerger, R. Campbell, and C. Somerville.** 1995. TOM a new aromatic degradative plasmid from *Burkholderia* (*Pseudomonas*) *cepacia* G4. Appl. Environ. Microbiol. 61:1352–1356.
477. **Shih, K. L., and J. Lederberg.** 1976. Effects of chloramine on *Bacillus subtilis* deoxyribonucleic acid. J. Bacteriol. 125:934–945.
478. **Silva, J., Jr.** 1994. *Clostridium difficile* nosocomial infections-still lethal and persistent. Infect. Control Hosp. Epidemiol. 15:368–370.
479. **Silver, S., and S. Misra.** 1988. Plasmid-mediated heavy metal resistances. Annu. Rev. Microbiol. 42:711–743.
480. **Silver, S., G. Nucifora, L. Chu, and T. K. Misra.** 1989. Bacterial ATPases: primary pumps for exporting toxic cations and anions. Trends Biochem. Sci. 14:76–80.
481. **Silvernale, J. N., H. L. Joswick, T. R. Corner, and P. Gerhardt.** 1971. Antimicrobial actions of hexachlorophane: cytological manifestations. J. Bacteriol. 108:482–491.
482. **Scott, E. M., and S. P. Gorman.** 1991. Glutaraldehyde, p. 596–614. *In* S. S. Block (ed.), Disinfection, sterilization and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.
483. **Spicher, G., and J. Peters.** 1976. Microbial resistance to formaldehyde. I. Comparative quantitative studies in some selected species of vegetative bacteria, bacterial spores, fungi, bacteriophages and viruses. Zentbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt 1 Orig. Reihe B 163:486–508.
484. **Spicher, G., and J. Peters.** 1981. Heat activation of bacterial spores after inactivation by formaldehyde. Dependence on heat activation on temperature and duration of action. Zentbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt 1 Orig. Reihe B 173:188–196.
485. **Springthorpe, V. S., J. L. Grenier, N. Lloyd-Evans, and S. A. Sattar.** 1986. Chemical disinfection of human rotaviruses: efficacy of commercially-available products in suspension tests. J. Hyg. 97:139–161.
486. **Springthorpe, V. S., and S. A. Satter.** 1990. Chemical disinfection of virus-contaminated surfaces. Crit. Rev. Environ. Control 20:169–229.
487. **Srivastava, R. B., and R. E. M. Thompson.** 1965. Influence of bacterial cell age on phenol action. Nature (London) 206:216.
488. **Srivastava, R. B., and R. E. M. Thompson.** 1966. Studies in the mechanism of action of phenol on *Escherichia coli* cells. Br. J. Exp. Pathol. 67:315–323.
489. **Stewart, G. S. A. B., S. A. A. Jassim, and S. P. Denyer.** 1991. Mechanisms of action and rapid biocide testing. Soc. Appl. Bacteriol. Tech. Ser. 27:319–329.
490. **Stewart, G. S. A. B., K. Johnstone, E. Hagelberg, and D. J. Ellar.** 1981. Commitment of bacterial spores to germinate. A measure of the trigger reaction. Biochem. J. 198:101–106.
491. **Stewart, M. H., and B. H. Olson.** 1992. Physiological studies of chloramine resistance developed by *Klebsiella pneumoniae* under low-nutrient growth conditions. Appl. Environ. Microbiol. 58:2918–2927.
492. **Stickler, D. J., B. Thomas, J. C. Clayton, and J. A. Chawla.** 1983. Studies on the genetic basis of chlorhexidine resistance. Br. J. Clin. Pract. Symp. Suppl. 25:23–28.
493. **Stickler, D. J., J. Dolman, S. Rolfe, and J. Chawla.** 1989. Activity of some antiseptics against urinary *Escherichia coli* growing as biofilms on silicone surfaces. Eur. J. Clin. Microbiol. Infect. Dis. 8:974–978.
494. **Stickler, D. J., J. Dolman, S. Rolfe, and J. Chawla.** 1991. Activity of antiseptics against urinary tract pathogens growing as biofilms on silicone surfaces. Eur. J. Clin. Microbiol. Infect. Dis. 10:410–415.
495. **Stickler, D. J., and P. Hewett.** 1991. Activity of antiseptics against biofilms

of mixed bacterial species growing on silicone surfaces. Eur. J. Clin. Microbiol. Infect. Dis. 10:416–421.
496. **Stickler, D. J., and B. J. King.** Intrinsic resistance. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
497. **Storz, G., and S. Altuvia.** 1994. OxyR regulon. Methods Enzymol. 234:217–223.
498. **Sutton, L., and G. A. Jacoby.** 1978. Plasmid-determined resistance to hexachlorophene in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 13:634–636.
499. **Sykes, G.** 1939. The influence of germicides on the dehydrogenases of *Bact. coli*. 1. The succinic acid dehydrogenase of *Bact. coli*. J. Hyg. 39:463–469.
500. **Sykes, G.** 1970. The sporicidal properties of chemical disinfectants. J. Appl. Bacteriol. 33:147–156.
501. **Takayama, K., and J. O. Kilburn.** 1989. Inhibition of synthesis of arabinogalactran by ethambutol in *Mycobacterium smegmatis*. Antimicrob. Agents Chemother. 33:1493–1499.
502. **Tattawasart, U., J.-Y. Maillard, J. R. Furr, A. C. Hann, and A. D. Russell.** 1997. Basis of the resistance of *Pseudomonas stutzeri* to antibiotics and biocides. Poster presented at Society for Applied Microbiology, Autumn Meeting.
503. **Taylor, D. M.** Inactivation of unconventional agents of the transmissible degenerative encephalopathies. *In* A. D. Russell, W. B. Hugo, and G. A. J. Ayliffe (ed.), Principles and practice of disinfection, preservation and sterilization, 3rd ed., in press. Blackwell Science, Oxford, England.
504. **Taylor, G. R., and M. Butler.** 1982. A comparison of the virucidal properties of chlorine, chlorine dioxide, bromine chloride and iodine. J. Hyg. 89:321–328.
505. **Tennent, J. M., B. R. Lyon, M. T. Gillespie, J. W. May, and R. A. Skurray.** 1985. Cloning and expression of *Staphylococcus aureus* plasmid-mediated quaternary ammonium resistance in *Escherichia coli*. Antimicrob. Agents Chemother. 27:79–83.
506. **Tennent, J. M., B. R. Lyon, M. Midgley, J. G. Jones, A. S. Purewal, and R. A. Skurray.** 1989. Physical and chemical characterization of the *qac*A gene encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. J. Gen. Microbiol. 135:1–10.
507. **Thomas, S., and A. D. Russell.** 1974. Studies on the mechanism of the sporicidal action of glutaraldehyde. J. Appl. Bacteriol. 37:83–92.
508. **Thomas, S., and A. D. Russell.** 1974. Temperature-induced changes in the sporicidal activity and chemical properties of glutaraldehyde. Appl. Microbiol. 28:331–335.
509. **Thurmann, R. B., and C. P. Gerba.** 1988. Molecules mechanisms of viral inactivation by water disinfectants. Adv. Appl. Microbiol. 33:75–105.
510. **Thurmann, R. B., and C. P. Gerba.** 1989. The molecules mechanisms of copper and silver ion disinfection of bacteria and viruses. Crit. Rev. Environ. Control 18:295–315.
511. **Trevor, J. T.** 1987. Silver resistance and accumulation in bacteria. Enzyme Microb. Technol. 9:331–333.
512. **Trujillo, P., and T. J. David.** 1972. Sporistatic and sporicidal properties of aqueous formaldehyde. Appl. Microbiol. 23:618–622.
513. **Trujillo, R., and N. Laible.** 1970. Reversible inhibition of spore germination by alcohols. Appl. Microbiol. 20:620–623.
514. **Tyler, R., G. A. J. Ayliffe, and C. Bradley.** 1990. Viricidal activity of disinfectants: studies with the poliovirus. J. Hosp. Infect. 15:339–345.
515. **Uhl, S.** 1993. Triclosan-resistant *Staphylococcus aureus*. Lancet 342:248.
516. **Vaara, M.** 1992. Agents that increase the permeability of the outer membrane. Microbiol. Rev. 56:395–411.
517. **Vaara, M., and J. Jakkola.** 1989. Sodium hexametaphosphate sensitizes *Pseudomonas aeruginosa*, several other species of *Pseudomonas*, and *Escherichia coli* to hydrophobic drugs. Antimicrob. Agents Chemother. 33:1741–1747.
518. **Van Cuyck-Gandre, H., G. Molin, and Y. Cenatiempo.** 1985. Étude de la résistance plasmidique aux antiseptiques. Mise au point de méthodes. Pathol. Biol. 33:623–627.
519. **Van Klingeren, B., and W. Pullen.** 1993. Glutaraldehyde-resistant mycobacteria from endoscope washers. J. Hosp. Infect. 25:147–149.
520. **Viljanen, P.** 1987. Polycations which disorganize the outer membrane inhibit conjugation in *Escherichia coli*. J. Antibiot. 40:882–886.
521. **Vischer, W. A., and J. Regos.** 1973. Antimicrobial spectrum of Triclosan, a broad-spectrum antimicrobial agent for topical application. Zentbl. Bakteriol. Mikrobiol. Hyg. Abt Orig. A 226:376–389.
522. **Waaler, S. M., G. Rolla, K. K. Skjorland, and B. Ogaard.** 1993. Effects of oral rinsing with triclosan and sodium lauryl sulfate on dental plaque formation: pilot study. Scand. J. Dent. Res. 101:192–195.
523. **Waites, W. M., and C. E. Bayliss.** 1979. The effect of changes in spore coat on the destruction of *Bacillus cereus* spores by heat and chemical treatment. J. Appl. Biochem. 1:71–76.
524. **Walker, J. F.** 1964. Formaldehyde. ACS Monogr. Ser. 3. Reinhold Publishing, New York, N.Y.
525. **Wallhäusser, K.** 1984. Antimicrobial preservatives used by the cosmetics industry, p. 605–745. *In* J. J. Kabara (ed.), Cosmetic and drug preservation. Principles and practice. Marcel Dekker, Inc., New York, N.Y.
526. **Walsh, S., J.-Y. Maillard, and A. D. Russell.** 1997. Effects of testing method on activity of high level antibacterial disinfectants. Poster presented at Society for Applied Microbiology Autumn Meeting.
527. **Walters, T. H., J. R. Furr, and A. D. Russell.** 1983. Antifungal action of chlorhexidine. Microbios 38:195–204.
528. **Wang, P., and H. E. Schellhorn.** 1995. Induction of resistance to hydrogen peroxide and radiation in *Deionococcus radiodurnans*. Can. J. Microbiol. 41: 170–176.
529. **Warth, A. D.** 1988. Effect of benzoic acid on growth yield of years differing in their resistance to preservatives. Appl. Environ. Microbiol. 54:2091–2095.
530. **Wheeler, P. R., G. S. Besra, D. E. Minnikin, and C. Ratledge.** 1993. Inhibition of mycolic acid biosynthesis in a cell-wall preparation from *Mycobacterium smegmatis* by methyl 4-(2-octadedylcyclopropen-1-yl)butanoate, a structural analogue of a key precursor. Lett. Appl. Microbiol. 17:33–36.
531. **White, D. C.** 1997. Antifungal drug resistance in *Candida albicans*. ASM News 63:427–433.
532. **Williams, N. D., and A. D. Russell.** 1992. The nature and site of biocide-induced sublethal injury in *Bacillus subtilis* spores. FEMS Microbiol. Lett. 99:277–280.
533. **Williams, N. D., and A. D. Russell.** 1992. Increased susceptibility of injured spores of *Bacillus subtilis* to cationic and other stressing agents. Lett. Appl. Microbiol. 15:253–255.
534. **Williams, N. D., and A. D. Russell.** 1993. Injury and repair in biocide-treated spores of *Bacillus subtilis*. FEMS Microbiol. Lett. 106:183–186.
535. **Williams, N. D., and A. D. Russell.** 1993. Revival of biocide-treated spores of *Bacillus subtilis*. J. Appl. Bacteriol. 75:69–75.
536. **Williams, N. D., and A. D. Russell.** 1993. Revival of *Bacillus subtilis* spores from biocide-induced injury in germination processes. J. Appl. Bacteriol. 75:76–81.
537. **Williams, N. D., and A. D. Russell.** 1993. Conditions suitable for the recovery of biocide-treated spores of *Bacillus subtilis*. Microbios 74:121–129.
538. **Wimpenny, J., W. Nichols, D. Stickler, and H. Lappin-Scott.** 1994. Bacterial biofilms and their control in medicine and industry. BioLine, Cardiff, Wales.
539. **Winquist, L., U. Rannug, A. Rannug, and C. Ramel.** 1984. Protection from toxic and mutagenic effects of hydrogen peroxide by catalase induction in *Salmonella typhimurium*. Mutat. Res. 141:145–147.
540. **Wood, P., M. Jones, M. Bhakoo, and P. Gilbert.** 1996. A novel strategy for control of microbial biofilms through generation of biocide at the biofilm-surface interface. Appl. Environ. Microbiol. 62:2598–2602.
541. **Wright, A. M., E. V. Hoxey, C. J. Soper, and D. J. G. Davies.** 1997. Biological indicators for low temperature steam and formaldehyde sterilization: effect of variations in recovery conditions on the response of spores of *Bacillus stearothermophilus* NCIMB 8224 to low temperature steam and formaldehyde. J. Appl. Microbiol. 82:552–556.
542. **Wu, Z. C., and X. J. Jiang.** 1990. The effects of chlorine disinfection on the resistance of bacteriophage f2 in water. Chung Hua Yu Fang I Hsueh Tsa Chih 24:196–198.
543. **Yakabe, Y., T. Sano, H. Ushio, and T. Yasunaga.** 1980. Kinetic studies of the interaction between silver ion and deoxyribonucleic acid. Chem. Lett. 4: 373–376.
544. **Yamamoto, T., Y. Tamura, and T. Yokota.** 1988. Antiseptic and antibiotic resistance plasmid in *Staphylococcus aureus* that possesses ability to confer chlorhexidine and acrinol resistance. Antimicrob. Agents Chemother. 32: 932–935.
545. **Yasuda-Yasuki, Y., S. Namiki-Kanie, and Y. Hachisaka.** 1978. Inhibition of germination of *Bacillus subtilis* spores by alcohols, p. 113–116. *In* G. Chambliss and J. C. Vary (eds.), Spores VII. American Society for Microbiology, Washington, D.C.
546. **Young, D. C., and D. C. Sharp.** 1985. Virion conformational forms and the complex inactivation kinetics of echovirus by chlorine in water. Appl. Environ. Microbiol. 49:359–364.
547. **Zavriev, S. K., L. E. Minchenkova, M. Vorličková, A. M. Kolchinsky, M. V. Volkenstein, and V. I. Ivanov.** 1979. Circular dichroism anisotrope of DNA with different modifications at N7 of guanine. Biochim. Biophys. Acta 564: 212–224.